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THE DIAGNOSTIC AND THERAPEUTIC USE OF UVEAL PIGMENT IN INJURIES OF THE UVEAL TRACT AND SYMPATHETIC OPHTHALMIA

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In a former paper by one of us (Woods [1]), clinical and experimental studies on the immune reactions following injuries to the uveal tract were presented. These studies showed that: (1) In an intra-ocular injury involving the uveal tract, where normal healing took place without the occurrence of a sympathetic disturbance in the fellow eye, substances developed in the blood serum which gave a positive complement fixation reaction with an antigen made from the pigment of the uveal tract. (2) In the cases of the intra-ocular injury involving the uveal tract when normal healing was delayed, and where sympathetic disturbance in the second eye sometimes occurred, this complement fixation reaction to pigment antigen was absent. Furthermore, there was not only the absence of this complement fixation reaction, but in one case of sympathetic disturbance there appeared to be a definite hypersensitiveness to pigment. Evidence was also presented which had led the author to believe that the occurrence of this complement fixation reaction was evidence of the development of an immunity by the organ-

ism to the pigment, and gave definite protection against the outbreak of a sympathetic disturbance in the fellow eye. Likewise, it seemed clear that the failure to develop this complement fixation reaction was evidence of the failure to develop an immunity, and was so attended by a persistance of inflammatory symptoms and the liability to the development of a sympathetic disturbance in the fellow eye.

The possible clinical use of this phenomenon for the purpose of early information as to the prognosis, in any given case of intra-ocular injury involving the uveal tract, is at once evident. Should these facts hold true, the development in the blood serum of a positive complement fixation against pigment antigen would warrant the surgeon in giving a favorable prognosis, and allow him to leave the injured eye without fear of the development of a sympathetic disturbance in the fellow eye. On the other hand, the failure to develop a positive reaction would give definite information to the surgeon that sympathetic ophthalmia was an event to be feared, and would allow removal of the injured eye before the outbreak of the sympathetic disease.

Likewise, the possibility for the therapeutic use of uveal pigment in a sympathetic ophthalmia is manifest. As much as the hypersensitivity predisposes to the outbreak of the disease and an immunity protects against the disease, as soon as a hypersensitivity is demonstrable, the self-evident course is to de-sensitize the patient, and then, as a therapeutic measure, proceed either to active or passive immunization.

It is the purpose of this paper to report cases of intra-ocular trauma involving the uveal tract, in which this serum complement fixation reaction against uveal pigment was used as a diagnostic procedure to determine the status of the case with regards to a possible outbreak of sympathetic ophthalmia. Further, one case of malignant sympathetic ophthalmia is reported in which uveal pigment was used as a therapeutic procedure.

The Diagnostic Use of the Serum Complement Fixation Reaction against Uveal Pigment

The sera from seventeen cases of intra-ocular injury involving the uveal tract, occurring in the private and dispensary practices of the authors, were examined in the complement fixation reaction against an antigen of uveal pigment. The technique of the reactions and the preparation of the antigen have been previously reported.[2]

Grouping these cases according either to the results of the reaction or to the clinical picture and result, they fall into three rather general groups. The first group comprises ten cases which gave a positive complement fixation reaction, and in which healing took place without the occurrence of any sympathetic disturbance. In three of these cases, however, for one or another reason enucleation was performed. The second group comprises three cases which all showed negative complement fixation reactions and at the same time showed more or less severe signs of a sympathetic disturbance. The third group comprises three cases which showed alarming symptoms in the injured eye, but without any manifestation of sympathetic disturbance in the second eye, and gave negative reactions.

Group I.—The following reports are of the cases in which the blood serum showed a positive complement fixation reaction against pigment antigen, following wounds of the uveal tract.

No. 1 (A.)—Struck in the left eye April 16, 1921 by a piece of steel; the wound of entrance was at the lower corneal margin. The splinter of steel passed back through the iris and was localized by the X-ray, in the region of the lower ciliary processes. Size of splinter, 12x2 mm. Removed by magnet extraction April 17, 1921. Serum reaction against pigment antigen on May 2, 1921 was + + + and on May 21, 1921 + + +. The injured eye gradually underwent pthisis. In March 1922, the injured eye was small, sightless, but not inflamed. Right eye held 20/15 vision throughout and has remained free from trouble to date.

No. 2. (O.)—Struck in right eye by a flying piece of steel on June 13, 1921. Wound of entrance at corneo-scleral border. The X-ray showed an intra-ocular splinter of steel, 13x3 mm. Successfully removed by magnet extraction on June 14, 1921. Serum reaction against pigment antigen on June 25, 1921, + + +. The injured eye healed quickly and retained 20/40 vision. The left eye held normal vision and has shown no subjective or objective signs of disturbance.

No. 3. (J.)—Struck in right eye on Sept. 14, 1921. Perforating wound of cornea with prolapse of iris and ciliary body. Prolapsed tissue excised within three hours after accident, scleral suture and conjunctival flap made. Uneventful healing, but a pthisical eye resulted. Serum reaction on Oct. 1, 1921, + + +. There was no disturbance of any kind in the left eye, but on Oct. 26, 1921, the right eye was enucleated for cosmetic reasons.

No. 4. (B.)—Struck in right eye Dec. 14, 1921; the patient's spectacles were broken. There was a perforating wound of the right eye, involving cornea, iris, lens, and ciliary body. Prolapsed tissue excised after accident. Traumatic cataract developed and was removed.

The eye showed a continual cyclitis of varying intensity, which, finally, starting about the middle of February, gradually subsided with a retention of 20/50 vision, with correction.

The following were the serum reactions in this case:—Dec. 14, 1921, ±; Jan. 9, 1922, + +; Jan. 21, 1922, negative. Feb. 13, 1922, + + +; March 27, 1922, + + +.

Vision in the uninjured eye was 20/15 at the time of the accident. There has at no time been any failure of the visual acuity or field or disturbance of any kind.

This case is of especial interest in that recovery was delayed, and with this delay the patient passed from a positive phase to a negative phase and, finally, to a second positive phase which he has steadily maintained.

No. 5. (B.)—November 1921, while hunting was struck in right eye with spent shot. Treated at home.

Exam. X-ray: Bullet down and in. Iridocyclitis and detached retina below.

Treatment: Enucleation. 12-11-21.

12-20-21. Discharged. Normal recovery.

12-11-21. Serum reaction against uveal pigment + +.

No. 6. (McC.)—Nov. 15, 1921. Struck in left eye with a piece of glass.

Exam: ×-shaped, penetrating wound in temp. side over region of ciliary body, the wound being 4-6 mm. long (Nov. 16, 1921).

Treatment: Scleral wound sutured—conjunctival flap.

Course: Looked very well for first ten days, then eye became red—ciliary congestion—iris showed greenish discoloration—Vit. exudate. Tension —.

12- 9-21. Enucleation.

12-16-21. Discharged. Right eye normal.

Dec. 15, 1921. Serum reaction against uveal pigment, ±.

No. 7. (R.)—Dec. 21, 1921, while playing, was struck in eye by a knife.

Exam: Perforating wound of cornea starting a few millimetres above limbus at 12 o'clock and running downwards for about 8 mm. Iris cut above. Some vitreous in upper part of wound. Lens swollen.

Treatment: 12-28-21. Adm. to hospital. Wound cleaned up and some of cataractous lens removed.

Course: 1-6-22. Serum reaction against uveal pigment negative; no immunity.

2- 4-22. Serum reaction against uveal pigment + + +.

2-17-22. Discharged. Ran through a long drawn out iridocyclitis with remissions and relapses. Finally left eye nearly white. Considerable cortex remaining. Capsule adherent to cornea and iris to capsule. V: R, 20/20; L, H. M.

3-20-22. Iridectomy.

4-15-22. Discharged. Normal recovery.

No. 8. (M.)—1-19-22. While on street was struck by some unknown object in right eye.

Exam: Linear incision through cornea from 9 to 5 o'clock and extending into ciliary body. Iris prolapsing, also part of ciliary body and some vitreous. V: L. P.

Treatment: Prolapsing iris, ciliary tissue, and vitreous, abscised. Wound closed with scleral and conjunctival flap.

Course: Normal recovery. Discharged 2-7-22. Vision 20/100. When last seen in clinic V. 20/50 +. Eye was white. No pain, left eye normal. V: 20/30.

Serum reactions against uveal pigment:

2- 1-22. Negative.

2-13-22. Anti-complementary.

2-28-22. +.

No. 9. (P.)—Sept. 1921, struck in right eye with splinter of steel. Three weeks ago noticed right pupil larger than left.

Exam: Corneal scar lower nasal quadrant with laceration of iris and opacity of lens directly behind. Embedded in retina, three disc diameters below disc there is an oblong-shaped foreign body surrounded by degenerated retina and choroid. Numerous small retinal exudates surrounding foreign body. V: 20/30.

Treatment: Giant magnet extraction through zonule and anterior cornea.

Course: 2-11-22. Serum reaction against uveal pigment, +.

2-17-22. Vitreous hazy. Some œdema and cloudiness about old choroidal scar.

2-25-22. Discharged. V: 20/70. Eye white. Vit. clearer. Fundus unchanged.

3-16-22. Readmitted. Detachment of retina below. V: R, 10/200; L, 20/20.

Left eye normal.

No. 10. (P.S.)—January 2, 1922, left eye injured by wire in coasting. Prolapsed iris abscised. Eye has remained red. Shrunken. No pain.

Seen on February 6, 1922. Ciliary congestion. Iris discolored. Scar in cornea on temporal periphery. Somewhat indrawn. Beginning phthisis, faulty projection.

Serum reaction February 13, 1922, + + +.

No. 11. (B.)*—Feb. 2, 1922. Was struck in right eye by nail. He withdrew nail following accident.

Exam: V: H. M. Perforating wound of cornea in lower nasal quadrant. Iris cut and caught in wound. Lens cataractous. P. L., good.

Treatment: 2-6-22. Wound freed of iris.

Course: 2-14-22. Anterior synech. at site of injury, some exudate back of lens. Eye very red.

2-25-22. Serum reaction—negative. No immunity.

3-15-22. Discharged. Eye still very red. Tension normal. V: 10/200; L. proj., N; lens subluxated and cataractous.

4-18-22. Eye still red. No pain. Tension normal. V: H. M.; L. eye normal.

GROUP II.—The following reports are of cases in which following injuries of the uveal tract, the blood serum showed negative complement fixation reactions. These cases showed either at the time of serum reactions, or developing later, disturbances in the second eye which were believed to be sympathetic in nature.

No. 12. (H.)—Shot in head Aug. 30, 1921, with a shot-gun. Many shot penetrated skull and one penetrated left eye, passing through eye-ball and lodging near nasal wall of orbit. Craniotomy was performed Sept. 3, 1921 with removal of many shot. Iridectomy was performed on left eye on Sept. 9, 1921, for removal of iris tissue incarcerated in wound of entrance. Serum reaction on Sept. 8, 1921, negative. Serum reaction on Sept. 30, still negative. The eye was still inflamed, and sightless. Enucleation was advised and refused. The child returned to his home in Virginia on Oct. 1, 1921. On Oct. 7, an attack of photophobia with pericorneal flushing in the right eye was reported by the local physician. The child was brought back to Baltimore on Oct. 14, 1921. At that time there was still decided photophobia, but the eye was otherwise objectively negative. Serum reaction on Oct. 14, 1921, was negative. Enucleation of left eye was performed on Oct. 16, 1921. Following enucleation, the photophobia of the right eye disappeared, and the eye has remained entirely normal since them.

No. 13. (H.)—Penetrating wound of left eye in 1909. Normal healing with retention of 20/70 vision. In May, 1921, some solder flew up into left eye causing great pain which steadily persisted. Right vision began to fail in July, 1921. On examination, Aug. 23, 1921, the left eye showed a displaced pupil, back of which, bulging the iris forward, a foreign body could be distinctly seen. There was a low grade cyclitis; tension normal, vision 20/70. The right eye showed nothing objectively except marked vascular congestion of the fundus and slight photophobia. Vision was 20/30—with correction. Visual fields showed normal field in right eye and concentric contraction in left. A complete medical study at Johns Hopkins Hospital showed only negative results. Laryngological and all other special examinations were likewise negative. On Aug. 26, 1921, the serum reaction with pigment antigen was negative. A tentative diagnosis of sympathetic irritation in the right eye was made and enucleation of the left eye advised. The patient returned to his home in North Carolina where enucleation was performed, and he reported that following enucleation all symptoms in right eye cleared.

No. 14. (H.)—This case of malignant sympathetic ophthalmia was later treated by de-sensitization and immunization with uveal pigment. Full details are reported below. The first serum reaction on Dec. 21, 1921, at the height of the disease, was negative.

* Case incomplete—no serum reaction has been done since negative reaction of Feb. 25th. Case is still under observation, and is included in this series, inasmuch as the report includes all cases tested up to March 15, 1922.

GROUP III.—The following reports are of three cases in which the serum reaction was negative and in which the injured eyes were enucleated as a precautionary measure.

No. 15. (B.)—Struck in left eye with a bit of steel Dec. 12, 1920; penetrating wound through ciliary body with intraocular retention of piece of steel. Removed by magnet extraction on the same day. Eye was sightless, and showed continual low grade cyclitis with periodic violent relapses. All medical examinations were negative. Radiograph showed no further intra-ocular foreign body. Jan. 17, 1921, and Feb. 15, 1921, the serum reaction against pigment antigen was negative. Left eye was enucleated on Feb. 21, 1921. Right eye normal.

No. 16. (K.)—In Nov. 1911 was struck by a tree branch and suffered from perforating injury for which he was treated in his home town until Jan. 13, 1922.

Exam: Marked pericorneal congestion. Perforating corneal wound just below center. Iris greenish and adherent to wound. Pupil occluded. P: L., good. V: L., 20/20.

Treatment: Enucleation with fat implantation, 1-14-22.

Course: 1-26-22. Discharged. Normal recovery. V: L., 20/20. Serum reaction against uveal pigment, 1-20-22, negative.

No. 17. (P.)—Struck in right eye with B.B. shot May 14, 1921. Shot penetrated globe through upper lid through sclera above cornea.

Treatment: Adm. to hospital and discharged as soon as hæmophthalmos had cleared up (6-9-21).

Course: Came to clinic for observation. Had several intraocular hæmorrhages. The tension gradually became soft and the eye remained red. Light projection faulty.

1-6-22. Serum reaction against uveal pigment, negative.

1-10-22. Adm. to hospital. Enucleation. Shot found outside globe, but adherent to it near optic nerve.

When last seen (4-18-22) left eye normal; V: 20/20.

There were two other cases in which all the serum reactions were negative, but these cases are of academic interest only. They were both instances of old sympathetic ophthalmia which had finally subsided following enucleation of the exciting eye. One case had followed sub-conjunctival scleral rupture and showed a sympathetic ophthalmia in the second eye which gradually recovered after enucleation of the injured eye. It is of interest that this patient showed a positive intradermal tuberculin reaction, confusing the diagnosis. The second case was one of delayed sympathetic ophthalmia in the left eye, following a bayonet wound of the right eye. When seen one year later, the left eye was nearly sightless. The right eye had been enucleated at the time of the sympathetic disturbance in the left eye.

COMMENT

In these cases above reported, it is frankly admitted that the presence or absence of a positive complement fixation reaction was not the sole index which governed the decision as to whether or not an injured eye should be left or enucleated. In two cases enucleation was performed before the blood was taken for the serum reaction, which in both cases was found positive. In other cases the presence of a positive serum reaction led to the temporary postponment of enucleation, which was later permanently abandoned on account of the favorable outcome of the case. Nevertheless, an analysis of those cases which gave positive results, excluding the two cases in which the picture is confused owing to enucleation having formed prior to the discovery of a positive blood, shows a rather significant fact. Had we relied, in these cases, upon a positive serum reaction as the index of when not to remove an injured eye, our faith would have been justified. In other words, the occurrence of a positive serum reaction, irrespective of the condition of the injured eye at the time the reaction was done, was always followed in time by a subsidence of inflammation without any manifestation of sympathetic disturbance.

Whether the opposite of this is true—that a negative reaction is sufficient indication for removal of an injured eye for fear of a possible sympathetic ophthalmia—is not so clear. Of the seven patients who showed negative reactions, one is still under observation and three at the time of the serum test already showed, or quickly developed, signs of a sympathetic disturbance. In the remaining three cases, we did not wait for a possible sympathetic disturbance to develop—the injured eyes were enucleated. But these three cases all showed a long-standing, chronic cyclitis, the eyes clinically appearing dangerous. So in all three enucleation was justified by the clinical symptoms alone.

In the absolute interpretation of negative results, other factors must be considered. In the first place, the blood serum practically never becomes positive before ten days after the injury, and frequently never before four weeks. Furthermore, as is illustrated by case No. 4, Group I, the patient may go from a positive to a negative phase, and then finally to a positive phase. If a single or two negative reactions be taken as a definite indication for the removal of an injured eye, eyes may be needlessly removed, for in a few weeks more it is possible that the patient may go into a permanent positive phase, and be automatically protected against sympathetic ophthalmia. Therefore, if the serum reaction is negative, the time interval after injury must be first considered. However, if in six weeks after injury the patient has failed to develop a positive reaction, it seems unlikely, from the information we now have, that a positive reaction will ultimately develop. In such cases the possibility of sympathetic ophthalmia, and enucleation of the injured eye should be carefully considered.

There is one further point to be considered in the interpretation of positive results. Is the immunity indicated by a positive reaction permanent, or is the immunity likely to fail and the patient later become susceptible to sympathetic ophthalmia? This question is very difficult to answer. We have seen one, and only one, case go from an initial positive phase to a negative phase, and this patient later, within three weeks after entering the negative phase, again became positive, and has so remained.

An immunological fact is known which may be somewhat analogous to this reaction. In an animal experimentally immunized by repeated injections of foreign protein, each fresh injection is followed by a temporary decrease in the antibody titer. If the original titer is not very high, this decrease may throw the animal from a positive to a negative phase. This reaction is followed, however, by a further increase over the original titer, so that the animal soon again becomes more positive than before.

Every other case examined which showed a positive reaction, so far as our work has gone, has remained positive. Yet the following fact is true. In the earlier work on man with this complement fixation reaction, most of the patients tested had a traumatism that was one year or more old; and it must be admitted that positive reactions in these were weaker than in the more recent cases. From a review of the work previously reported, it seems clear that immunity, once definitely established, will last for from two to three years, and probably much longer, but gradually will become weaker.

Summarizing the results shown by the cases here reported which showed positive reactions, considered in the light of our previous studies, it seems probably that a positive reaction indicates a definite immunity to the development of sympathetic ophthalmia and that this immunity will last at least several years, if not permanently. From these findings it seems, then, that with the occurrence of a positive serum reaction, unless the clinical condition clearly contra-indicated such a course, the surgeon is warranted in not enucleating injured eyes.

The Therapeutic Use of Uveal Pigment

The basic points thus far emphasized in our work have been these: (1) The outbreak of sympathetic ophthalmia seems to be dependent upon an existing hyper-sensitiveness to uveal pigment.[1] (2) The occurrence of an immunity against uveal pigment protects against the development of sympathetic ophthalmia. If these two fundamental points hold true, as it appears they do, they point at once to two very definite procedures—preventive and therapeutic—as regards sympathetic ophthalmia: (1) As a preventive or prophylaxis against sympathetic ophthalmia, the obvious procedure is to remove the hypersensitivity and produce the desired immunity. (2) As a therapeutic measure, to remove the one factor which appears essential to the outbreak of sympathetic ophthalmia, and to substitute for it the one factor that appears to make sympathetic ophthalmia impossible; in other words, again to remove the hyper-sensitiveness and produce an immunity to uveal pigment.

With the use of uveal pigment as a prophylactic measure we have had no experience. The case reported below is also the only instance in which we have used the pigment as a therapeutic agent. It may be stated frankly that, inasmuch as this work is scarcely out of the experimental stage, we felt considerable reluctance in using uveal pigment in man, knowing that a hypersensitivity to it existed, unless the circumstances surrounding the case were such as to make any procedure, offering the least hope, justifiable. The case below reported more than satisfied these conditions. Before any injections were made, Professor Hans Zinsser was good enough to consult with us, and it was only after this final consultation that the procedure was undertaken.

Report of a Case of Sympathetic Ophthalmia

The patient, a boy aged 8, seen on September 10, 1921, had an unhealed corneal ulcer, with prolapse of the iris in a dense scar, occupying the lower half of the right cornea, the result of a gonorrheal conjunctivitis, which had begun three months before. Vision was reduced to light perception and the tension was increased. The attempt to preserve the eye seemed justified; on September 13th, an iridectomy was done upward, the defect in the cornea was curetted, the prolapsed iris was removed as well as possible and the corneal wound covered with a conjunctival flap. This healed and the patient returned to his home after two weeks with vision of 2/200.

He returned on October 31st, because the other (left) eye had become red three days before. The left eye showed slight ciliary congestion, posterior corneal deposits, posterior synechiæ. V: 20/20. Tension, normal. Interior, normal. The right eye was white. V: 2/200. The area of the corneal defect covered by a conjunctival flap was depressed and ocular tension was reduced.

There was no history of indigestion or overeating; the urine was normal; the stool showed evidence of intestinal fermentation, and the patient was placed on a sugar and starch-free diet and high colon irrigations. The eye was given the usual treatment. November 7th, V: 20/40; vitreous hazy; retinal veins engorged. November 9th, right eye enucleated. Serum reaction against uveal pigment was negative, showing no immunity whatever. A large cyanide subconjunctival injection was given; pilocarpine sweats and mercurial inunctions were begun. November 14th, atropin eczema. Left pupil contracting; nodules appeared in iris. Leucocytes, 3000; no lymphocytosis. December 8th, V: 20/40. Deep ant. chamber. Iris flat with nodules. Optic nerve blurred, retina hazy. Nonspecific protein therapy was tried; a course of typhoid vaccines was given by Dr. Bonime. Though these were followed by the usual reactions, the leucocyte count did not increase and the eye continued to grow steadily worse. There was a marked follicular conjunctivitis with pseudomembranes. The general treatment was then changed to salicylate of soda in large doses. The eye showed more and more the characteristic changes of sympathetic ophthalmia with nodules in the iris; peripheric retraction of iris; complete synechiæ and capsular opacity in the lens. On Jan. 5, 1922 the injections of uveal pigment were

commenced. During the period of treatment with the pigment, the progress of the eye disease did not seem to be particularly changed. Anterior chamber shallow; pupil contracting; iris uneven, flat total adhesion; tension increased. The last pigment injection was given Jan. 12.

January 18, 1922.—The eye was better; cornea and iris were clearer. T. 36. This improvement was only of brief duration and an exacerbation occurred on February 2, 1922, when the eye became more red, harder, and there was more exudation in the anterior chamber and in the iris. On Jan. 20, 1922, eight days after the course of immunization had been completed, the serum reaction had become + + —almost complete fixation. Following the exacerbation of Feb. 2, the eye gradually improved, though the opacity of the lens remained about stationary; iris retracted with some anterior peripheric adhesions; capsular opacities. On the last examination, the tension was normal and the eye white, V: 20/200. Details in fundus not visible. The blood examination on Feb. 8 gave + + +, showing complete fixation.

Technique of Treatment

Procedure with uveal pigment.—The suspension of pigment used in the case was made up as before described(2) and preserved with 0.15 per cent tri-cresol. As the pigment is de-naturized by heat, unheated pigment was given, the only step taken for sterilization being the addition of tri-cresol, which is sufficient to destroy all ordinary non-spore forming pathogenic organisms. Since the critical condition of the patient did not allow sufficient time for animal experiments to exclude the remote possibility of the presence of tetanus spores in the preparation of pigment, it was decided to administer tetanus anti-toxin as a prophylactic measure. A precautionary intra-dermal test was made with tetanus anti-toxin to determine whether the patient was sensitive to the serum. This was positive, and he was first desensitized with serum in the usual manner, and then given 1500 units of tetanus anti-toxin. An intra-dermal skin test with uveal pigment was then made.

Technique of Intra-dermal test (Jan. 6, 1922).—Three different dilutions of the normal pigment suspension (the pigment of the uveal tract of an eye of a cow to 7.5 c.c. of salt solution is called "normal,") were used. The solutions were 1:100, 1:50, and 1:10. "normal," were used. The solutions were 1:100, 1:50, and 1:10. Through a fine hypodermic needle small intra-dermal injections, sufficient to raise a bleb about one half the diameter of a dime, were made with each dilution on the flexor surface of the forearm. A control was made with 0.15 per cent tri-cresol in normal salt solution. At the end of one hour the three blebs where the pigment suspension had been injected were surrounded by a striking, confluent urticarial wheal, whereas the control was practically negative. Within six hours this urticarial reaction had disappeared. Since the patient was strikingly hypersensitive to uveal pigment, we at once proceded with desensitization.

Technique of desensitization (Jan. 6, 1922).—Intra-muscular injections in the buttock were made at two-hour intervals. The so-called "normal" suspension was used for the first four doses. The amounts given were 1.0 c.c.; 1.5 c.c.; 2.0 c.c. and 2.5 c.c. For the fifth injection a suspension of twice normal strength was used, 2.5 c.c. (the equivalent of 5.0 c.c. of the normal) were given. There was no essential change in either the blood pressure, pulse, temperature or respiration during this period of desensitization. The only change observed was a generalized urticaria with the resultant unpleasant itching. This was relieved by a 70 per cent alcohol rub, and entirely passed within sixteen hours.

Technique of immunization.—On Jan. 6, during desensitization, a total of 12 c.c. of the normal suspension had been given, the last injection being the equivalent of 5.0 c.c. of the normal. On Jan. 9, the equivalent of 7.5 c.c. of the normal (condensed in a bulk of 2.0 c.c.) were given by intra-muscular injection into the buttock. On Jan. 12, after a second three-day interval, the final injection, the equivalent of 10 c.c. of the normal, was given by intra-muscular injection into the buttock. As for the previous injection, the pigment was condensed in a total volume of 2.0 c.c. No untoward symptoms accompanied either of the latter injections.

Comment

Sympathetic inflammation after perforating ulcers is rare. Peters comments on the fact that sympathetic ophthalmia has been frequently observed after perforation of a corneal ulcer complicating a gonorrheal process and concludes that the conditions in gonorrheal inflammation are more favorable to a development of sympathetic ophthalmia than in other keratitides. As an operation was performed in some of these cases, it may be that the prolapse is not so dangerous as the operation which was undertaken to remove it. The operations performed in these cases (quoted from Peters) were excision of the prolapse, (Schieck), cauterization (Trousseau, Gifford and Fuchs), abscission of corneal staphyloma and conjunctival suture (Fuchs).

In the case just described there seemed to be a definite intestinal autotoxemia. No change in diet or treatment, however, was of any avail. There was no lymphocytosis. Recent investigations have not shown the occurrence of a lymphocytosis in sympathetic ophthalmia. Treatment with typhoid vaccines was without avail. A leucocytosis could not be obtained.

Enucleation after the onset of the sympathetic ophthalmia was, as usual, without influence on the course of the irido-cyclitis in the remaining eye.

Microscopic examination of the eye first affected showed a characteristic diffuse infiltration of the iris and the adjoining iris angle, with mononuclear lymphocytes, a moderate infiltration of the ciliary processes and neighboring vitreous body, and only a few scattered foci in the choroid.

In view of the fact that partial recoveries have been observed in sympathetic ophthalmia, irrespective of the form of treatment, the inflammation having finally exhausted itself, it would be unwise to draw any deductions from a single experience. Yet in this case, after desensitization and immunization, the morbid process, with one slight and short period of exacerbation, came to a standstill; the eye became white and free from irritation and so remained.

SUMMARY

The immune reaction associated with intra-ocular injuries involving the uveal tract of the eye was made use of in seventeen such cases as a diagnostic procedure. In ten cases the complement fixation reaction was positive. These cases showed a normal healing without the occurrence of any sympathetic disturbance. One case is still incomplete. Three cases showed negative reactions, and of these one showed, clinically, a malignant sympathetic ophthalmia, and two showed definite signs of sympathetic irritation. The three remaining cases showed negative reactions and in these cases the injured eyes were enucleated as a precautionary measure. Two other cases of old sympathetic ophthalmia showed negative reactions.

The one case of malignant sympathetic ophthalmia showed, in addition to a negative blood picture, a positive skin reaction to the intradermal injection of pigment. In this case uveal pigment was used as a therapeutic agent. The patient was first desensitized to pigment and then actively immunized, with apparently beneficial results.

This case of sympathetic ophthalmia occurred, in a boy aged 8, after an operation for a perforated corneal ulcer with prolapse of iris after a gonorrheal ophthalmia. The inflammation pursued a steadily progressive course with all the symptoms of the severe type of sympathetic disease, despite all usual treatment—diet, intestinal irrigations, pilocarpin sweats, mercury inunctions, large doses of sodium salicylate, and non-specific protein therapy. Finally, following desensitization and immunization with uveal pigment, the process came to a stop, the eye became white, free from inflammation, the tension fell to normal, and has so remained. The process continued to be active for three months but has now been stationary for two months. Vision: 6/60. Peripheric retraction and flat total adhesion of iris; capsular opacities. Tension, normal.

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OBSERVATIONS ON THE PRODUCTION OF TOXIC SUBSTANCES BY PNEUMOCOCCI

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INTRODUCTION

In spite of the advances that have been made in our knowledge of the etiology and epidemiology of pneumococcus lobar pneumonia during the past decade, it must be admitted that but little progress has been effected in our understanding of the pathological physiology of the infection or of the factors that are responsible for the chain of events characterized by pneumococcus infection in man and to which we give the name lobar pneumonia. The common explanation for many of the events that take place during the course of lobar pneumonia is the assumption that along with the initiation of an inflammatory reaction in the lungs a substance or several substances are formed which are capable of being transported by the lymph or the circulating blood to distant portions of the body, there to bring about alterations in structure or function of vital organs. It is difficult to explain many of the phenomena of pneumonia without resort to this assumption, and it has therefore gained considerable favor with those who have observed the disease in man. That there is a profound alteration in the function of organs at some distance from the primary focus is not to be denied. Furthermore, the time-honored observations that the magnitude of these alterations is often out of proportion to the extent of the pathological process, that they so often disappear abruptly at an appreciable interval of time before the local pathological process gives evidence on physical examination of beginning to clear up and while viable pneumococci may still be present in the lung tissue,[1] all support the conception that in pneumonia one has to deal with a toxæmia as well as a local infection and at times a bacteræmia.

The conception of the existence of a toxæmia in pneumonia is an attractive one, because it fits in so well with what one observes in the pneumonia patient, and is a relatively simple hypothesis. When, however, one attempts to test the validity of this hypothesis in the light of what is definitely known about pneumococcus infections, one realizes at once how difficult it is to bring forward in support of such a view evidence of an experimental nature which is at all convincing. It is impossible to say at the present time whether or not the pneumococcus through its own metabolic activity and independently of the host produces a toxin or toxins, or whether such a substance or substances are the joint product or products of a reaction between the parasite on the one hand and the cells of the host on the other. The latter conception has of recent years been gaining ground with some students of the infection.

Historical

Attempts to isolate toxic substances from pneumococci have been manifold. The earlier workers, following the lead given by the work on diphtheria toxin, sought for poisonous substances in the fluid portion of cultures of pneumococcus. The reported results are somewhat conflicting but in the main negative. Kruse,[2] in 1910, and Neufeld and Handel,[3] in 1912, after a review of the earlier literature came to the conclusion that active poisons had not been obtained from the pneumococcus which could with any assurance be regarded as being identical with those which are believed to be present in pneumococcus infection in man and to give rise to the symptoms of general intoxication. Wadsworth[4] concluded from his experiments that "dead pneumococcus culture material does not contain the active poisons formed in infection by living pneumococci."

Following Friedberger's work on the production of "bacterial anaphylatoxin" in the test-tube Neufeld and Dold[5] produced a similar substance, using pneumococcus as antigen, and these same workers[6] showed later that by extracting pneumococci in salt solution containing 0.1% lecithin toxic substances could be obtained, which, upon intravenous injection into guinea-pigs, resulted in death with symptoms of anaphylaxis. About the same time Rosenow,[7] by extraction of pneumococci in salt solution for forty-eight hours at 37° C., secured solutions which on intravenous injection into guinea-pigs gave similar results. Cole[8] worked with solutions obtained by dissolving pneumococci in sodium cholate. With such solutions injected intravenously into guinea-pigs he obtained anaphylactic-like symptoms including death with pathological findings similar to those encountered in serum anaphylaxis, and in the test-tube he was successful in producing lysis of red blood cells. Similar results were obtained by him with solutions of pneumococci obtained by freezing and grinding the bacteria. The hemolytic action of solutions of pneumococci could be inhibited by a serum prepared by injecting the solution into sheep and rabbits, as well as by minute amounts of cholesterin. Cole regarded the substances derived from pneumococci by procedures calculated to disrupt the cell as existing preformed in the bacterial cell and not as being the product of digestion as ordinarily understood by that term. He would class these substances as endotoxins.

S. Solis-Cohen, Weiss and Kolmer[9] repeating Cole's experiments were "unable to produce uniformly a toxin which was hemolytic and also produced anaphylaxis on primary intravenous injection in guinea-pigs." Weiss,[10] however, was able to produce it in sufficient quantities to arrive at the conclusion that it is very labile, that it is a true protein, that it inhibits the agglutinating action of antipneumococcal serum but is itself not affected by antipneumococcus serum, and that upon intracutaneous injection into sensitized guinea-pigs it produces an erythema and hemorrhagic edema in the subcutaneous tissue overlying the muscle. From our own experience in working with sodium cholate solutions of pneumococci we can testify to the difficulty of securing a toxin from pneumococci by this method with any great degree of regularity. Because of the inconstancy of the results we were forced to abandon our attempts to study this substance.

The search for pneumococcus toxin has not been confined solely to the study of the organism itself. It is but natural that poisonous substances should be sought for in material derived from the pathological process as well. Issaeff[11] found that filtered blood of infected animals as well as blood or peritoneal exudate sterilized by heating at 58° C. for two hours was toxic in amounts of one to two per cent of body-weight. The Klemperers[12] succeeded in killing young rabbits with the blood serum of infected rabbits in doses of two per cent of the body-weight. F. Klemperer[13] found that the filtrate of the fresh pleural exudate of a dog was toxic for other dogs. Kruse and Pansini[14] were not able to demonstrate toxic substances in the blood of infected animals, nor was Cole.[15] The latter, however, found that the peritoneal exudates obtained from guinea-pigs dying after an intraperitoneal injection of pneumococci contained substances, which on intravenous injection into normal guinea-pigs gave rise to immediate symptoms like those seen in anaphylaxis and killed about one third of the animals with anaphylactic shock. Rosenow and Arkin,[16] using extracts of pneumonic lungs and injecting normal dogs intravenously, produced changes which they regarded as similar to those seen in dogs dying after anaphylactic shock. Weiss, Kolmer and Steinfield[17] investigated the toxicity of pneumonic lungs, and by subjecting the pathological tissue to pressure in a hydraulic press succeeded in obtaining a juice which, when sterilized by the addition of phenol or by heating to 56° C. for thirty minutes, was two to five times as toxic for mice, guinea-pigs and rabbits as was a juice obtained from normal human lungs treated in the same manner. Extracts of consolidated lungs in which the consolidation was produced by the intra-bronchial injection of aleuronat were in the main more toxic than extracts of normal lung and less toxic than those of pneumonic lungs. These extracts of pneumonic lungs were also found to be lytic for red blood cells. Manipulations, such as heating, drying and filtration through Berkefeld filters, decreased the toxicity of these extracts. It should be pointed out that the differences in toxicity of the extracts of pneumonic lungs and those of normal lungs were not so great as entirely to exclude the experimental error resident in all biological experimentation.

Discussion

One may summarize the previous work on this phase of pneumococcus infection as follows: There is general agreement amongst the workers in this field that pneumococci or pathological material the result of pneumo-

coccus infection can be so manipulated as to yield (somewhat irregularly) substances which upon intravenous injection into laboratory animals give rise to symptoms and lesions which are identical with those observed after a second dose of protein-containing material has been administered to an animal previously sensitized to that protein. The correct interpretation of these facts is not so easy; their relation to pneumococcus infection in man is by no means clear, as Cole and others have pointed out. There is no direct evidence to show that the toxic substances obtained by manipulating the penumococcal cells or exudate produced by them are ever formed in the human body during the course of pneumococcus infection. If it is true that they are formed solely as a result of solution of the bacterial cell, and if they are produced during the course of pneumonia and are responsible for the general toxic symptoms encountered in that disease, we must suppose that many pneumococci are destroyed in the lung during the course of pneumonia and even at the outset of the disease, for it is well known that the toxic symptoms of pneumonia are often quite severe at that time as well as later. To what extent extracellular destruction of pneumococci takes place in the consolidated lung is likely to remain a matter of conjecture and not susceptible of determination. Whether or not such a process does occur, it is certain that many of the organisms are taken up by the leucocytes and destroyed within the cells, and it is difficult to see how this intracellular process of destruction could result in the setting free of pneumococcus endotoxin unless the leucocytes can excrete such a substance without alteration. The work on agar anaphylatoxin and particularly the studies of Novy and his associates [18] seem to us to do away with the idea that the animal effects observed after injection of pneumococcus toxin are specific or characteristic, no matter whether the toxin is obtained from the pneumococci themselves or from pathological exudates. In view of their work the query may be raised whether the occurrence of anaphylactic symptoms in guinea-pigs after intravenous injection is a sufficiently safe criterion for the determination of the toxicity of bacterial preparations. Further evidence of toxicity, such as might be elicitated by subcutaneous or intraperitoneal injection, would seem to us desirable before concluding that one was dealing with a toxin capable of being formed in man in amounts sufficient to give symptoms. It should be noted here that Weiss, Kolmer and Steinfield [19] obtained deaths after intraperitoneal injection of the exudate from pneumonic lungs in amounts considerably less than those needed if cultures were used in the preparation of the toxin. It will be generally admitted that the toxic substances thus far described are of a low potency for the customary laboratory animals. This fact is evidence for the view that such substances play a slight, if any, rôle in the pneumococcus infection in man, although it is possible that a toxin of a low potency operating over a prolonged interval of time may be just as deleterious as one of high potency acting over a short period.

It has seemed to us much more reasonable to associate the phenomena of intoxication in pneumonia with the growth of pneumococci rather than with their death and dissolution. When one examines the peritoneal exudate of mice dying after an intraperitoneal injection of living pneumococci, one sees evidence of tremendous proliferation of the bacteria and, as judged by the Gram stain, almost all appear to be in a healthy condition. Such mice invariably give a positive heart's blood culture. This same picture, moreover, is seen when mice are injected with sputum containing many other bacteria beside pneumococci. In such instances the pneumococci rapidly outstrip the other bacteria in their growth (frequently the latter are in great part destroyed) and one sees an overwhelming infection with pneumococci as if conditions were ideal for the growth and multiplication of those bacteria and not of the others. One certainly does not get the impression that pneumococci have been destroyed in great numbers in the peritoneal cavity of the mouse, and yet the mouse shows symptoms of intoxication and ultimately succumbs. It is difficult to conceive of rapid growth and rapid extra-cellular death of pneumococci occurring at the same time and in the same place, where conditions are as favorable for growth as they appear to be in the peritoneal cavity of mice.

If the toxic phenomena of pneumonia were associated with the growth and multiplication of pneumococci, it seemed to us conceivable that toxic substances might be present in a culture of pneumococcus while the organisms were growing rapidly, and that the chemical nature of such substances might be altered during the later stages of growth of the culture so that they would no longer be recognizable as such. In some studies on the nature of bacterial lag one of us [20] had been impressed by the fact that, when pneumococci are grown in meat infusion broth at 37° C., the maximum number of bacteria is attained within eighteen hours as a rule, sometimes earlier, and that after this period there is often a falling off in the number of living bacteria. The period during which pneumococci multiply at the maximum rate of growth in favorable fluid media is relatively short, not longer than twelve hours as a rule, and during this time significant changes take place in the H-ion concentration of the medium.[21] It seemed to us that this might constitute a desirable time at which to search for pneumococcus toxin, since the more acid condition of the medium later on might bring about destruction of the relatively unstable substance formed during an earlier phase. Support for this view is offered by the work of De Kruif and Ireland,[22] who have shown that the hemolysin produced by the streptococcus hemolyticus reaches its maximum at a very early period in the life of the culture, begins to disappear when the period of active growth ceases, and may disappear completely in fourteen hours. Zinsser and his

associates[23] have recently reported finding substances toxic for rabbits in cultures of a number of different species of bacteria and have found that the potency of these substances, while admittedly low, was in many instances greater in the case of young cultures, and appeared to diminish with age. They failed to find indication of the formation of such substances in the case of pneumococcus, type I, or staphylococcus aureus "in a few isolated experiments."

Because of the importance of the problem and because we have been interested in the question for several years, it seems to us desirable to extend these observations on the pneumococcus, making use not only of the ordinary fluid media but also of substances such as human serum and human blood, in order to approach as closely as possible conditions in the body.

Most workers in bacteriology have in the past dealt with cultures as old as or older than eighteen hours. They have lost sight of the fact that in fluid cultures of actively growing organisms the maximum number of bacteria is usually attained by that time, and that one is no longer dealing with a culture the members of which are multiplying actively. Chemical conditions in a culture of that age are quite different from those obtaining a very short while afterward.[24] To test the assumption that there might be found in fluid cultures of pneumococci, during the period of active growth, substances of a toxic nature, a series of experiments were carried out. It may be said in advance that they yielded only negative results. For that reason the results will not be given in detail, but only a general statement of the method and findings will be submitted for purposes of record.

Technique

Two strains of pneumococci type I, isolated from recent cases of lobar pneumonia, were used. The media employed were: (1) beef infusion broth prepared according to the method described by Avery and Cullen;[25] (2) normal human serum; and (3) defibrinated human blood. The last two media were employed with the idea that conditions existing in the human body would be more closely approximated than in the case of beef infusion broth. Incubation was at 37° C. Specimens were removed at frequent intervals for filtration and plating for counts. Filtration was carried out with previously tested Berkefeld N candles. The clear filtrates were injected intraperitoneally into white mice in amounts of one cubic centimeter each. Bacterial counts served to show at what phase in the period of growth the specimens were removed.

Results

In no instance were any toxic effects observed in the mice, no matter whether the specimens were obtained from the culture at the beginning, during the middle or toward the end of the period of maximal rate of growth. Our experiments, therefore, were entirely negative so far as demonstrating the presence of a soluble toxin in the fluid portion of a rapidly growing culture of pneumococcus in beef infusion broth, normal human serum or defibrinated human blood. They cannot be said to throw any light on the complex problem of the cause of the intoxication in lobar pneumonia.

Summary and Conclusions

1. The relationship of the toxic substances obtained from pneumococci by solution of the bacterial cell or by manipulation of pneumonic exudates to the phenomena of intoxication in pneumonia in man is discussed.

2. Filtrates of actively growing cultures of pneumococci in beef infusion broth, normal human serum and defibrinated normal human blood are not toxic for mice when injected intraperitoneally.

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INFLUENZA-LIKE BACILLI

GROWTH OF INFLUENZA-LIKE BACILLI ON MEDIA CONTAINING ONLY AN AUTOCLAVE-LABILE SUBSTANCE AS AN ACCESSORY FOOD FACTOR

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For a long time, in spite of numerous studies, very little was definitely known about influenza bacilli. Practically every statement in regard to them has been challenged, leading to numerous controversies, one of which was whether they actually required hemoglobin for their growth. Pfeiffer [1] at one time thought that on a purely morphological basis he could distinguish pseudo-influenza bacilli from the true forms. Wolff,[2] in 1903, stated that Pfeiffer had decided his original idea of pseudo-influenza bacilli to be incorrect. Later, pertussis bacilli led to more confusion and some workers [3] speak of pertussis-like bacilli without describing them accurately enough for others to recognize these organisms when found. When the pandemic of influenza came in 1918, *B. influenzæ* was characterized as a Gram-negative, non-motile, pleomorphic, aërobic, hemoglobinophilic bacillus. All strains were considered more or less alike serologically, regardless of morphology or virulence.

Since 1918 much profitable work has been done on influenza bacilli. It is known that there are hemolytic and non-hemolytic varieties, that they usually reduce nitrates to nitrites, that some form indole while others do not, that some ferment no sugars while others attack various carbohydrates. The most interesting work, however, has been that dealing with the growth requirements [4-10] of these organisms. It has been definitely shown that true influenza bacilli require two accessory food substances, one of which resists autoclaving at 15 pounds for 30 minutes, while the other does not. Both substances are present in blood. They have been found elsewhere; the autoclave-stable factor in unfertilized eggs and potatoes, the autoclave-labile one in yeast extract, potatoes, tomatoes, beans and carrots. Respiratory strains differ culturally [11-13] and show a marked serologic heterogeneity.[14, 15] This difference is not so evident in the group producing meningitis.[16] Most of the meningitic strains examined were closely related culturally and serologically. *B. hemoglobinophilus canis*, described by Friedberger [17] and placed in the hemophilic group, has been shown [18] to require the addition of only the autoclave-stable substance as an accessory food factor. This bacillus has much in common with influenza bacilli, and several years ago it would not have been easy to differentiate it from them.

Has all this work made it possible to say that any given bacillus does or does not belong to the influenza group? Is there some one thing common to all of them that no other group possesses? Are there organisms found in throat and lung cultures that are not influenza bacilli, yet which might be mistaken for them? Many of these questions can be answered now. Pertussis bacilli when first isolated are rather difficult to grow. Special media have to be used, but after the strains have been isolated for some time neither of the food accessory factors are required. *B. hemoglobinophilus canis* requires the addition of only the autoclave-stable substance. Hemolytic and non-hemolytic influenza bacilli, after from 2 to 7 years' cultivation on artificial media, still need both accessory food substances. Certain bacilli to be discussed in this paper, after 2 years' cultivation outside the body, will grow on media to which only the autoclave-labile factor has been added as an accessory food substance. These bacilli might be mistaken easily for influenza or pertussis bacilli and probably have been so mistaken in the past.

DESCRIPTION OF TWO INFLUENZA-LIKE BACILLI

Bacillus No. 1 was isolated from a throat culture of a patient with influenza in February, 1920. Bacillus No. 2 was isolated from the right lung of a patient who died of pneumonia following influenza in February, 1920. The throat culture of this patient yielded hemolytic streptococci and hemolytic influenza bacilli. A culture from the trachea showed hemolytic streptococci, hemolytic influenza and non-hemolytic influenza bacilli. From the right lung hemolytic streptococci were recovered in addition to bacillus No. 2. The non-hemolytic influenza bacillus recovered from the trachea was a Gram-negative, non-motile, pleomorphic bacillus which formed indole, reduced nitrates to nitrites, fermented no sugars, produced little or no change in blood-milk, and required both accessory food factors for growth.

Growth on Blood-Agar.—These influenza-like bacilli grow somewhat more slowly on blood-agar than ordinary influenza bacilli, but the colonies are enough like those of the latter to be mistaken for them. This is especially true of No. 2. Young colonies of No. 1 are regular, round, glassy and transparent. Older colonies have a brownish granular center and occasionally very old colonies have a crinkled surface with radiating folds (Fig. I, 1a and 1b). The colonies of bacillus No. 2 are flatter, not so glassy, and have a more granular brownish center (Fig. I, 2). The colonies of No. 1 are usually smaller and not so moist as those of No. 2. The difference in size of

colonies is not always so marked as in the figures. Colonies vary from 1 to 3 mm. in diameter. Both bacilli grow well under aërobic conditions.

Morphology, Staining, Motility.—The organisms are Gram-negative, non-motile, pleomorphic, non-sporebearing bacilli which cannot be differentiated from influenza bacilli morphologically. As a rule No. 1 (Fig. II) is smaller and more regular than No. 2 (Fig. III).

Hemolysis.—Neither strain hemolyses red blood cells.

Production of Indole.—Neither bacillus forms indole.

Reduction of Nitrates.—Both bacilli reduce nitrates to nitrites.

Production of Amylase.—A starch-splitting ferment is not produced by either strain.

Action on Milk.—No marked change was noticed in the blood-milk tubes after incubation for 3 weeks.

Fermentation of Sugars.—The fluid and solid media for fermentation tests were prepared as described in a previous paper.[16] Dextrose, sucrose, maltose and xylose were not fermented in a liquid medium by either bacillus. On a solid medium No. 1 showed a questionable acid formation from dextrose, sucrose and maltose.

Growth Requirements.—The autoclave-stable and the autoclave-labile growth accessory substances were prepared and used as indicated in a previous paper.[18] The stable factor was hematin, the labile one was yeast extract sterilized by means of a Mandler filter.

The two influenza-like bacilli would not grow for more than a generation or two on meat extract agar, meat infusion agar, 2 per cent peptone agar, 2 per cent peptone agar and hematin, 2 per cent peptone water, 2 per cent peptone water and hematin, or 2 per cent peptone water and ascitic fluid. The results of these experiments are summarized in Table. I.

TABLE I.
Growth Accessory Requirements of Certain So-called Hemophilic Bacilli.

Bacterium	Meat Extract Agar	Meat Infusion Agar	2% Peptone Agar	2% Peptone Agar and Hematin	2% Peptone Agar and Yeast Extract	2% Peptone Water and Yeast Extract	2% Peptone Water and Autoclaved Yeast Extract	2% Peptone Agar and Hematin and Yeast Extract
B. pertussis No. 256	+	+	+	+	+	+	+	+
B. hemoglobinophilus canis	—	—	—	+	—	—	—	+
B. influenzæ, non-hemolytic	—	—	—	—	—	—	—	+
B. influenzæ, hemolytic	—	—	—	—	—	—	—	+
Influenza-like bacilli Nos. 1 and 2	—	—	—	—	+	+	—	+

+ Indicates that successful transplants were made as long as desired. — Indicates that successful transplants were not obtained, or for one or two generations only.

In meat infusion broth these influenza-like bacilli grow well for one generation; after that none of the tubes showed any gross turbidity. If, however, amounts of 1 c.c. were inoculated daily from tube to tube, transplants from the tubes to blood agar showed from 10 to 30 colonies regularly for many generations. Evidently there was a slight multiplication of the bacilli, or at least they did not die for a long time. This could not have been told from the gross appearance of the broth tubes and was discovered by making transplants to blood agar. The meat infusion broth was autoclaved. This discrepancy may be more apparent than real, for it may be possible for the heat-labile factor to withstand a short autoclaving without being entirely destroyed.

In Table II are listed the most important differential biological reactions of the hemophilic bacilli so far studied.

TABLE II.
Differential Biological Reactions of Certain So-called Hemophilic Bacilli

Bacterium	Reduction of Nitrates	Indole Production	Motility	Hemolysis of Red Blood Cells	Fermentation of Sugars	Action on Milk	Accessory Food Factors	
							Autoclave-Stable	Autoclave-Labile
B. pertussis not recently isolated	—	—	—	—	—	Strongly Alk.	—	—
B. hemoglobinophilus canis	+	+	—	—	+	Indefinite	+	—
Influenza-like bacilli Nos. 1 and 2	+	—	—	—	±	Indefinite	—	+
B. influenzæ hemolytic	+	±	—	+	±	Indefinite	+	+
B. influenzæ non-hemolytic	+	±	—	—	±	Indefinite	+	+

(Under nitrates) + indicates reduction, — indicates no reduction. (Under indole) — indicates no indole formation, + indicates indole formation, ± indicates indole may or may not be formed. (Under motility) — indicates lack of motility. (Under hemolysis) + indicates hemolysis takes place, — that hemolysis does not occur. (Under sugars) — indicates no sugars are fermented, + indicates that certain sugars are fermented, ± indicates that certain sugars may or may not be fermented. (Under food accessory factors) + indicates that the substance is essential, — indicates that the substance is not essential.

Discussion

In another paper[18] various ideas about the nature and the action of the two accessory food substances were discussed. At that time the question was raised as to whether there is an actual interaction between the two substances, as suggested by some observers, in promoting the growth of influenza bacilli. The question was raised then because *B. hemoglobinophilus canis* was found to require the addition of only the autoclave-stable factor. Evidence has been presented in this paper that certain influenza-like bacilli need the addition of only an autoclave-labile substance as an accessory growth factor. Yeast extract contains many other substances besides this heat-labile factor. It is not these substances, however, that are so essential because autoclaved yeast extract does not meet the needs of these bacilli whereas filter-sterilized extract does. It is fully realized here again

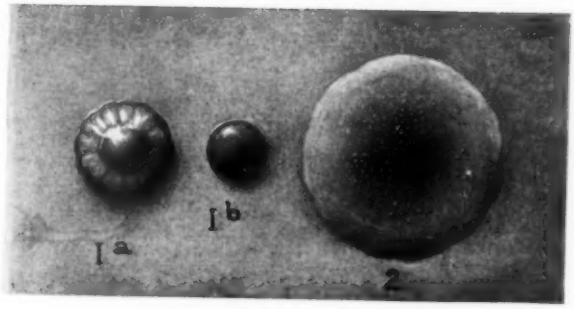

FIG. I

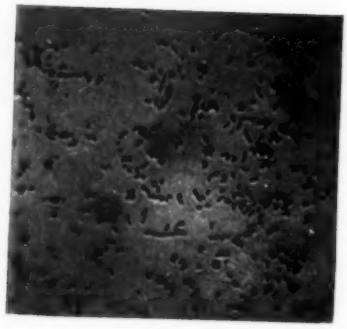

FIG. II

FIG. III

that one cannot argue conclusively by analogy from one group of organisms to another. Yet the fact that these influenza-like bacilli, as well as *B. hemoglobinophilus canis*, require only one of the accessory factors, casts further doubt upon the possibility of any interaction between the two accessory substances in promoting growth of true influenza bacilli.

Only two influenza-like bacilli have been described here; there are probably many more. From throat cultures taken during the epidemic of influenza in 1920 a number of atypical strains were obtained. Most of these were lost, as it is more difficult to cultivate the atypical strains than the typical ones on artificial media.

Stillman [19] and others have recovered more than one kind of influenza bacillus from the same patient. The influenza-like bacillus, No. 2, was obtained from the lung of a patient who had true non-hemolytic and hemolytic influenza bacilli in the trachea and hemolytic influenza bacilli in the throat culture. No. 2 was considered an influenza bacillus for a long time as it would not grow on meat infusion agar. It was only after working out these strains carefully, particularly in regard to their growth requirements, that the difference was discovered. Until a few years ago the criteria by which an organism was judged to be an influenza bacillus were: Gram-negative, non-motile, pleomorphic, aërobic, hemoglobinophilic bacillus. The hemoglobinophilic qualities were tested on ordinary nutrient agar. Various workers must have had some mental conception of a true influenza bacillus because they were always speaking of typical and atypical ones. Most of this differentiation was made upon morphology alone. It is evident that many mistakes were made.

Is it possible to identify a given bacillus as a true *B. influenzæ?* Although it may be impossible to give a final answer to this question, one is better qualified to discuss it now than three or four years ago. It seems advisable to determine the accessory food factor requirements of each suspected bacillus, and to call only those true influenza bacilli which require both factors. The ones which require the addition of only the heat-stable factor have been named already by Friedberger.[17] What, however, should be the designation of the influenza-like bacilli which need only the heat-labile accessory food factor as found in unautoclaved yeast extract? *Bacillus para-influenzæ* or *Hemophilus para-influenzæ* might serve as a good name for this group of organisms which are probably no more alike culturally or serologically than are the true influenza bacilli.

CONCLUSIONS

Two influenza-like bacilli have been described which require the addition of only an autoclave-labile substance as an accessory food factor.

Bacillus para-influenzæ or *Hemophilus para-influenzæ* has been suggested as the name for this group of organisms.

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EXPLANATION OF FIGURES

Fig. I.—1a, type of colony that occasionally appears in very old cultures of bacillus No. 1. 1b, usual type of colony in 48-hr. culture of bacillus No. 1. 2, usual type of colony in 48-hr. cultures of bacillus No. 2.

Fig. II.—Film from a 24-hr. culture of bacillus No. 1 on blood agar. (×1200.)

Fig. III.—Film from a 24-hr. culture of bacillus No. 2 on blood agar. (×1200.)

THE JOHNS HOPKINS HOSPITAL BULLETIN

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A NEW METHOD OF TESTING LIVER FUNCTION WITH PHENOLTETRACHLORPHTHALEIN

SECOND COMMUNICATION

By Sanford M. Rosenthal

(From the Pharmacological Laboratory, Johns Hopkins University)

In a previous paper [1] the writer described a method of testing liver function by injecting phenoltetrachlorphthalein intravenously and studying its rate of disappearance from the blood stream. This was carried out in normal dogs, and in those with damaged livers. Under normal conditions, if the dosage is not too large, the liver is practically the only organ that excretes the dye, as was first shown by Abel and Rowntree.[2] Later Rowntree, Bloomfield and Hurwitz,[3] and Whipple, Mason and Peightal [4] used phenoltetrachlorphthalein as a test for liver function by determining the amount excreted in the stools during the 48 hours subsequent to its injection intravenously. Further studies with this method were carried out by McLester and Frazier,[5] Kahn and Johnston,[6] Sisson,[7] Chesney, Marshall and Rowntree [8] and Krumbhaar.[9] Their reports show results that are conflicting, and this method has not met with extensive clinical usage. Certain of its disadvantages were stated previously.[1] In 1916 McNeil [10] introduced the method of testing liver function by determining the time required for the dye to appear in the bile, obtained through the duodenal tube. This procedure was given a recent trial by Aaron, Beck and Schneider [11] who used the Meltzer-Lyons method of bile drainage. Results by this method must be interpreted in terms of negative values; the test is not quantitative, and from experiments presented in this paper it is felt that the appearance time of tetrachlorphthalein in the bile is governed to a large extent by the condition of the bile-ducts, and does not necessarily depend upon the amount of functionating liver tissue present. By the author's method, results are quantitative, and, as is shown in the following experiments, they are within certain limitations independent of the condition of the bile channels; it is believed that the rate at which the liver removes this dye from the blood stream gives an index of the functional capacity of the entire organ.

When, owing to impaired function, the liver is unable normally to excrete this phthalein, it escapes in the urine, but does so with difficulty and quite slowly. This means of escape is apparently dependent upon the length of time that the dye remains in the blood stream, and upon the concentration that it reaches there. In the former experiments chloroform poisoning was used to produce liver injury;[12, 13, 14, 15] when the impairment of function was greatest, as evidenced by the degree of jaundice and the toxic condition of the dogs, remarkably high curves were obtained. During the stage of repair, the concentration that the dye reached in the plasma, and the time required for it to disappear from the blood stream, gradually decreased, until curves approaching normal were obtained after sufficient time had elapsed for restoration of function.

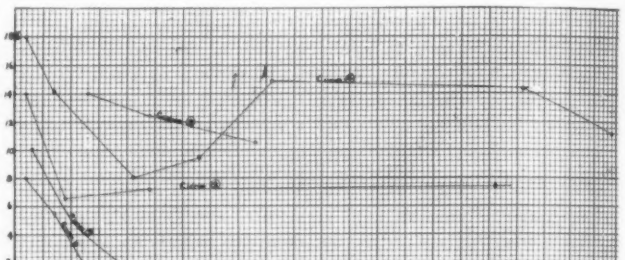

Fig. 1

Fig. 1 illustrates the very striking retention of the dye in the blood during the degenerative stage of chloroform poisoning. On the eleventh day of this experiment, although all objective signs and symptoms of impaired liver function had disappeared, the sensitiveness of the test is shown by the fact that the curve then obtained had not quite returned to normal.

Simplified Method for Quantitative Estimation

In these experiments the dose of tetrachlorphthalein employed was 5 mgs. per kilo. A sample specimen of blood was withdrawn before the injection; 2 c.c. of the plasma obtained were diluted with 5 c.c. of water, to which had been added a known quantity of the dye. This was compared colorimetrically with samples taken at frequent intervals following the injection. For convenience, results have been expressed in per cent of concentration, 10 mgs. of the dye to 100 c.c. of plasma being taken as 100-per-cent concentration. The technique was fully described in the former paper.

By this method, colorimetric readings below 10 per cent were often unsatisfactory, and hemolysis usually rendered comparison impossible. It was found that a series of standards could be made by adding concentrated solutions of the dye to the undiluted plasma, small test-tubes of uniform size being used. Eight cubic centimeters of blood are withdrawn before injection of the dye: standards are made from the plasma as follows:

TABLE I.

0.2 c.c. 100% dye sol. 0.6 c.c. plasma	0.2 c.c. 80% dye sol. 0.6 c.c. plasma	0.2 c.c. 60% dye sol. 0.6 c.c. plasma	0.2 c.c. 40% dye sol. 0.6 c.c. plasma	0.2 c.c. 20% dye sol. 0.6 c.c. plasma	0.2 c.c. 12% dye sol. 0.6 c.c. plasma
25%	20%	15%	10%	5%	3%

FIG. 2

The unknown plasma is alkalinized with 3 drops of 5-per-cent sodium hydroxide, and without dilution is matched directly with the standards, using naked eye comparison in a good light. Two or three cubic centimeters of blood yield sufficient plasma for comparison. High readings by this method check with the colorimetric method closely enough for practical purposes; low ones are usually more accurate, and also the presence of hemoglobin proves less confusing. However, unless the plasma is free from bile and hemoglobin, readings below 5 per cent have been recorded as large, medium, or faint trace, and charted between 0.5 and 3.0 per cent.

A simple qualitative test, that shows up very faint traces of the dye, has also been worked out. To unalkalinized plasma several drops of 3-per-cent hydrochloric acid are added; this is carefully layered over a 5-per-cent solution of sodium hydroxide in a small test-tube. At the point of contact a blue to red line (dependent upon the amount of dye present) appears. Hemoglobin gives a light yellow line, and bile has been found not to interfere. This test can be carried out on quantities of plasma sufficiently small to permit of blood collection in Wright's blood capsules.

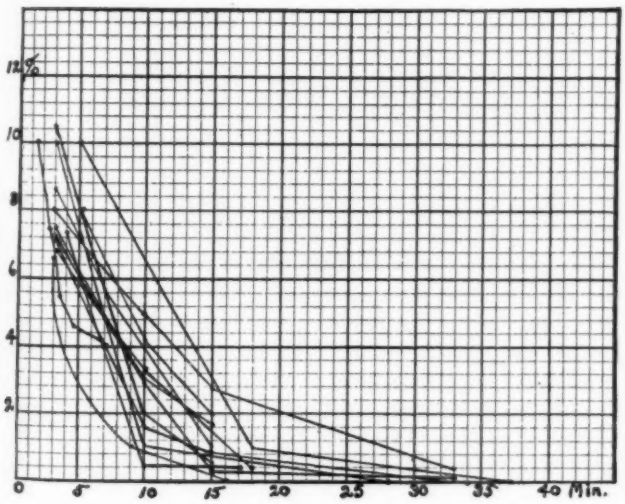

FIG. 3

NORMAL CURVES

With the dosage of 5 mgs. per kilo, there is a most obvious uniformity in the curves obtained. In a series of 12 normal dogs 13 curves varied only 4 per cent at their most divergent point (Fig.3). At 3 minutes 6 to 10 per cent concentrations were present, and at 15 minutes determinations ranged from a large trace (3 per cent) to complete disappearance of the dye. The "ring test" often remained positive for 30 to 45 minutes.

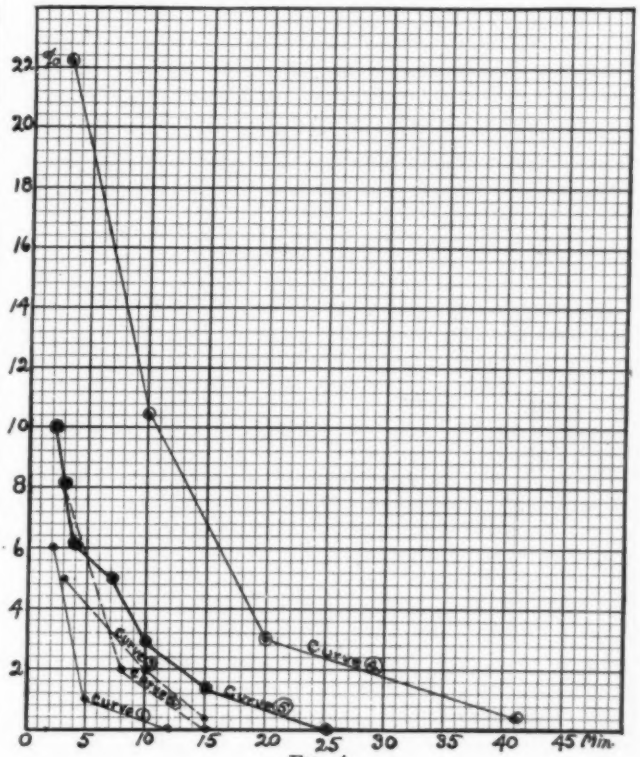

FIG. 4

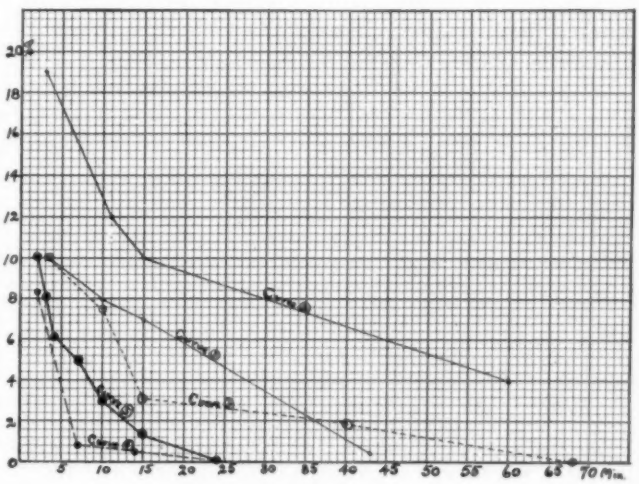

FIG. 5

VARIATION OF DOSAGE

To determine the influence upon the concentration that the dye reaches in the plasma and its rate of disap-

pearance, doses of the phthalein varying from 1.7 mgs. of 10.0 mgs. per kilo have been employed in several dogs (Fig. 4), and in one cat (Fig. 5). It is seen that the curves obtained bear a very definite relation to the amount used. It is also observed that with the usual (5 mgs. per kilo) dose, two cats (Fig. 5) showed somewhat higher curves, and one rabbit a slightly lower curve, than was obtained with the same dose in dogs. These experiments emphasize the importance of adhering to a fixed dosage per body weight, and also suggest that different curves might pertain for different species of animal.

Phosphorus Poisoning

Phosphorus, in large doses, causes widespread necrosis and autolysis of the liver parenchyma.[16, 17, 18] Pathologically, there is a close resemblance to the lesions found in acute yellow atrophy. In the earlier stages the liver is enlarged; the lobules are pale yellow in color, and the liver cells that remain alive are filled with fat. In non-fatal cases the organ soon decreases in size, and in the second week it becomes smaller than normal, microscopically showing connective-tissue proliferation, and often evidence of repair.[19, 20, 21]

Four dogs were given moderately large doses of phosphorus in oil, intra-muscularly. Prior to the injection they were starved for 24 hours, and following it they were kept upon a carbohydrate-free diet. The following protocol is included:

Dog 18. Male, weight 7.3 kilos. Light ether anesthesia while curves were obtained (See Fig. 6).

June 16: 36.5 mgm. tetrachlorphthalein injected into right external jugular. Curve 1 obtained. After experiment, 12 mgs. phosphorus in oil injected into gluteal muscles.

June 17, 18: Dog does not appear ill. No bile in urine.

June 19: 36.5 mgs. phthalein injected. No cholemia, no bile in urine. Curve 2 obtained.

June 20, 21: Animal is listless, and looks moderately toxic. Urine not dark, but contains bile.

June 22: Above dose of dye injected. Curve 3 determined. Moderate amount of bile in plasma and urine. Slight icteroid tinge to scleræ. Dog appears somewhat ill.

June 23 to 26: Dog brightened up considerably. Jaundice disappearing.

June 26: Normally active and playful. No icterus of scleræ. Urine not dark; trace of bile (Gmelin's test) in plasma and urine. Curve 4 obtained. Animal killed. On autopsy liver edge 2 cm. above costal margin. Color not abnormal. Some increase in connective tissue. No gross evidence of increase in fat. Microscopic examination showed a few scattered areas, chiefly in the centers of the lobules, where the liver cells had been replaced by connective tissue containing many leucocytes. These areas were interpreted as zones of focal necrosis, from which the necrotic cells had been removed, and in which almost complete repair had taken place.

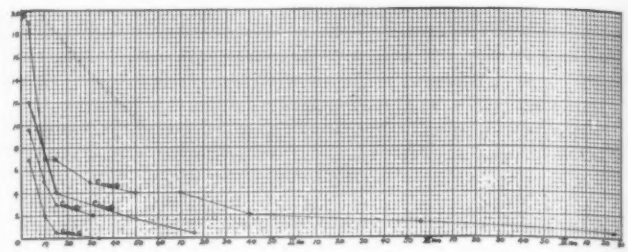

Fig. 6

When ether was employed, the injection of the dye was given within 15 minutes after the anesthetic was begun, so that the effect of ether upon liver function, as shown by Whipple,[22] could be neglected. Paraldehyde anesthesia was used during the experimentation upon dog 6. 15 mgs. of phosphorus in oil were given intramuscularly. Four days later the animal was quite ill; considerable bile was present in the plasma and urine; on this day a high curve was obtained (Fig. 7). The animal died while under anesthesia. Autopsy showed the characteristic liver changes of phosphorus poisoning.

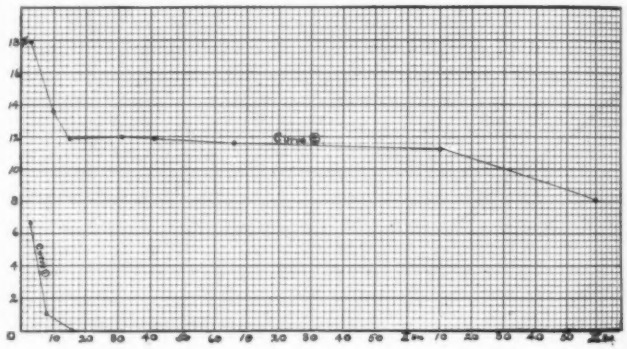

Fig. 7

Dog 14 (Fig. 8) became quite ill by the second day following the injection of 15 mgs. of phosphorus, although little bile was present in either the plasma or urine. A moderately high curve was found at this time; the low readings were rendered only approximately accurate because of hemolysis. The animal died at the end of the experiment.

The normal curve of dog 16 was obscured by lipemia. Forty-eight hours after the injection of 15 mgs. of phosphorus, the phthalein test was again made, and readings fell within the range of early abnormality; the animal did not appear ill. Five days after the injection, icterus was present, and the dog appeared to be in a toxic condition. A curve characteristic of moderately severe liver damage was found (Fig. 9). The animal was killed because of a septic condition of the neck; the liver presented typical gross changes of necrosis and fatty degeneration.

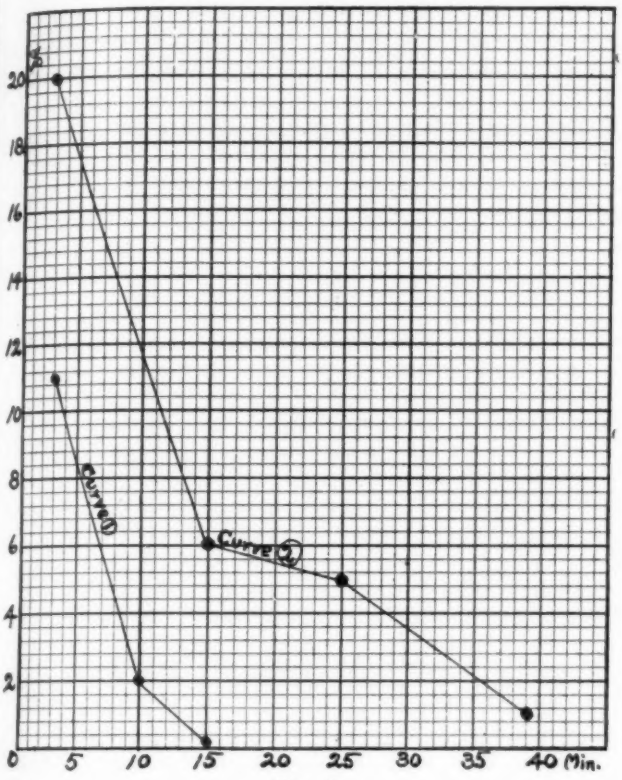

FIG. 8

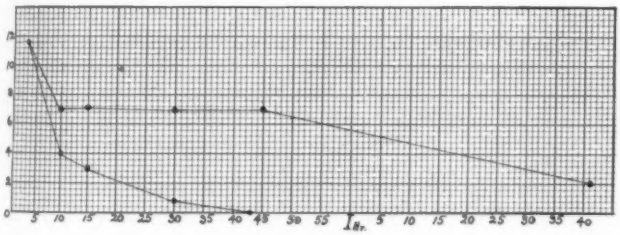

FIG. 9

LIGATION OF THE COMMON DUCT

In acute obstructive jaundice there is dilatation of the biliary passages behind the obstruction; the hepatic cells and cells of Kupfer become deeply pigmented, but there is very slight cell degeneration, except after prolonged obstruction.[23, 24, 25, 26, 27] The liver cells apparently continue their functions, including the excretion of bile, which is taken up by the blood capillaries and lymphatics.[28, 29, 23, 25]

It therefore seemed of interest to find out how rapidly the dye would be removed from the blood stream following ligation of the common bile duct. After the normal disappearance time had been determined, the common ducts of five dogs were ligated, and after allowing varying intervals of time to elapse, curves were again obtained. The striking fact was brought out, that in these animals the tetrachlorphthalein was removed from the blood stream in normal, or almost normal time, except that a trace of dye remained present in the blood as long as these experiments were continued.

TABLE II.

	Minutes	2	3	4	5	10	15	30	45	1 Hr.	2 Hrs.	3 Hrs.	4 hrs.
Dog No. 3*	Day before Ligation	15.			8.	T.	T.	T.					
	1 hour after	13.				T.	T.						
Dog No. 7	2 days before Ligation		10.			T.		O.					
	2 days after		12.5			3.9.	F.T.	F.T.	F.T.	F.T.			
Dog No. 10	3 hrs. before Ligation			7.3		F.T.	F.T.						
	2 days after		10.			F.T.	F.T.						
Dog No. 24	1 hour before Ligation		7.			3.	V.F.T.						
	2 days after		10.			5.	L.T.	L.T.	L.T.	L.T.	L.T.	L.T.	L.T.
		Per cent of dye in plasma											

* The high curves in Dog No. 3 are due to poisoning by chloroform administered a week previously.

FIG. 10

Only one hour had elapsed after ligation in dog 3, when the second test was made; there was no essential difference from the curve obtained previous to ligation (Fig. 10). No bile had appeared in the plasma or urine. Two days after ligation dogs 7, 10 and 24 received their second injections of the dye. The cystic ducts as well as the common ducts were ligated in dogs 10 and 24. All of these animals became icteroid. Curves very slightly higher than the normals for these dogs were reached in the second determinations, but the differences are negligible (Fig. 10).

A curve was obtained in dog 21 three days after ligation of the common duct (Fig. 11). It shows the practically normal rate of disappearance and the persistence of a trace of dye in the plasma is demonstrated, in that, twelve hours after this injection of dye, the "ring test" on the plasma was positive and at this time a large amount of phthalein was present in the urine. Twenty-four hours after the injection, the ring test on the plasma was just barely detectable, and the dye was still present in a catheterized specimen of urine. Following the injection of phthalein in these animals with obstructive jaundice, varying amounts appeared in the urine of all of them except dog 3, who was killed 45 minutes after the injection was made. The appearance of this dye in the urine during experimental obstructive jaundice has been previously studied by Whipple.[4]

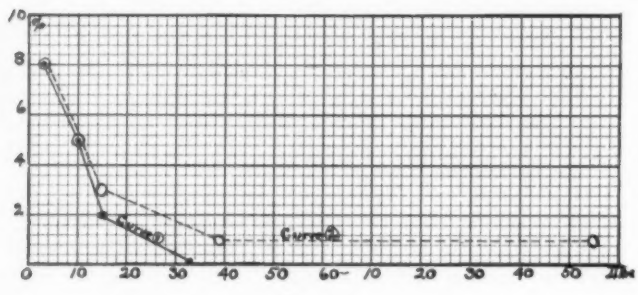

FIG. 11

It is felt that these curves are doubly significant. Firstly, it seems probable that the taking up of the dye by the liver cells, and its excretion in the bile, are independent functions, bearing in mind that chronic obstruction will undoubtedly produce cell damage. The former function seems to be dependent upon the extent and degree of degeneration of the liver cells. The latter function and the time of appearance of the dye in the bile would appear to be influenced greatly by the patency of the biliary passages, and would not necessarily be an index of the condition of the liver cells.

Secondly, while it is felt unwise to draw broad conclusions until the test has been given the trial of time and usage, the writer wishes to point out that, in these experiments, a method has been demonstrated that differentiates the early jaundice due to mechanical obstruction in which cell damage is slight, from the jaundice associated with degeneration of the hepatic epithelium. Experiments are being conducted with phenolsulphonphthalein to determine whether an analogous behavior occurs in renal conditions, in an attempt to develop a test for kidney function upon the same principle.

SUMMARY AND CONCLUSIONS

Phenoltetrachlorphthalein has been intravenously injected and its behavior in the blood stream has been studied in normal animals, in dogs following chloroform poisoning, phosphorus poisoning, and in dogs after ligation of the common and cystic bile-ducts.

The concentrations reached in the plasma of normal dogs, after 5 mgs. per kilo injections, are strikingly constant. Approximately 10 per cent is present in 2 minutes, 8 per cent in 3 minutes, and from a large trace to absence in 15 minutes. The rate of disappearance was found higher in two cats, and more rapid in one rabbit. Variation of the dose has a direct influence upon the curve obtained.

There is a marked increase in these concentrations, with a greatly prolonged rate of disappearance, after injury to the liver parenchyma; the extent to which this occurs depends upon the degree of damage to the liver cells, as evidenced by the condition of the animals, and by autopsy findings. Curves approaching normal are obtained if repair has taken place.

Curves have been obtained in jaundice due to mechanical obstruction that differ widely from those found in the jaundice associated with extensive degeneration of the hepatic cells.

A simplified method is herein presented for quantitative determination of the amount of dye in the plasma. A practical test for its qualitative detection is also described.

A clinical study is in progress, and a series of cases will be reported in the near future.

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LEGENDS

Fig. 1.—Dog No. 5. Chloroform Poisoning. May 5, normal curve (1). May 7, two hours and fifteen minutes chloroform anæsthesia. May 8, curve (2), eighteen hours later. Dog not very ill; chloroform again given for one hour. May 9, curve (3), second day. May 12, curve (4), fifth day. May 18, curve (5), eleventh day.

Fig. 2.—Table I. Preparation of series of standards to which specimens of undiluted plasma are compared directly. 10 mgs. of phenoltetrachlorphtalein to 100 c.c. of water used as 100 per cent solution of dye.

Fig. 3.—Curves of disappearance of phthalein from blood stream. 13 curves on normal dogs; 5 mgs. per kilo dosage.

Fig. 4.—Variation of the dosage of dye in normal dogs. Curve (1), Dog No. 11: 1.7 mgs. of tetrachlorphthalein per kilo. Curve (2) and curve (3), Dog No. 12 and Dog No. 13: 2.5 mgs. per kilo. Curve (4), Dog No. 15: 10 mgs. per kilo. Curve (5), composite curve constructed from Fig. 3, 5 mgs. per kilo.

Fig. 5.—Curves on normal cats and rabbit. Curve (1), Cat No. 2, 5 mgs. per kilo. Curve (2), Cat No. 2, 10 mgs. per kilo. Curve (3), Cat No. 3, 5 mgs. per kilo. Curve (4), Rabbit No. 1, 5 mgs. per kilo. Curve No. 5, composite curve from Fig. 3.

Fig. 6.—Dog No. 18. Phosphorus poisoning with convalescence. Curve (1), before intramuscular injection of 12 mgs. of phosphorus. Curve (2), three days after injection. No icterus; dog not ill. Curve (3), six days after injection. Bile in urine and plasma; moderately toxic. Curve (4), eleven days after injection. Dog appears normal; small trace of bile in urine.

Fig. 7.—Dog No. 6. 15 mgs. of phosphorus. Curve (1), normal. Curve (2), four days after injection. Animal quite ill; moderate jaundice.

Fig. 8.—Dog No. 14. 15 mgs. of phosphorus. Curve (1), before injection. Curve (2), two days later. Dog very toxic.

Fig. 9.—Dog No. 16. 15 mgs. of phosphorus. Curve (1), (above), two days after phosphorus injection. Curve (2), (below), five days after injection: dog moderately ill, slight jaundice.

Fig. 10.—Table II. Curves before and after ligation of common bile-duct.

Fig. 11.—Ligation of common bile-duct. Dog No. 21. Curve (1), before ligation. Curve (2), three days after ligation; deep jaundice.

A STUDY OF TRICHOMONAS HOMINIS, ITS CULTIVATION, ITS INOCULATION INTO ANIMALS AND ITS STAINING REACTION TO VITAL DYES

By MARY JANE HOGUE, PH. D.

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In a previous paper the author [1] has shown that a pure line of *Trichomonas hominis, i.e.,* a race started from a single individual, can be isolated, and that this pure line can be kept in culture, the medium best suited for this purpose being Locke-egg. In this medium the organism has to be transferred every two or three days, since the cultures reach their greatest development on the second or third day and rarely live more than five or six days.

In the fall of 1921 a very simple method was devised, by which *Trichomonas hominis* can be easily kept alive in test-tube cultures for thirty-five days and in some cases for over sixty-six days, without being transferred. This greatly facilitates the work of keeping cultures in stock for experimental work.

The stock, from which the pure line used for these experiments was isolated, has been cultured for eight months in this laboratory. The pure line was started when the stock was two and one half months old, and has been kept under constant observation for the past five and a half months.

Sodium chloride serum water medium. The method used is the following: To a flask containing 100 c.c. of 0.85 per cent sodium chloride solution, which has been sterilized in the autoclave, add 10 to 15 c.c. of sterile sheep serum water. For these animals sheep serum is better than pig serum, though the latter can be used. The serum water is prepared by diluting one part of sheep serum with three parts of distilled water, and is sterilized in the Arnold sterilizer, being heated at 100° C. for one hour on three successive days. This solution will then have a pH of 7 to 7.4. After thoroughly mixing, pour 15 c.c. of this solution into sterile test tubes and inoculate with *Trichomonas hominis* by means of a capillary pipette, the transferred material being deposited at the bottom of the test-tube. The surface of the medium is then covered with sterile paraffin oil to prevent evaporation and the tubes are incubated at 36° C.

The serum in the medium very soon coagulates, owing to the action of the bacteria which are growing in the culture with the *Trichomonas.* This coagulated material settles to the bottom. It is here that the organisms can be found in largest numbers.

Trichomonas hominis does not divide rapidly in these deep cultures covered with paraffin oil. In counting the organisms in one field (oc. 10, obj. 16 mm.) one rarely finds more than eight or ten individuals and usually there are not more than three or four in a field. At this slow rate of division the waste products do not accumulate rapidly and the culture lives for a long time.

When large numbers of *Trichomonas* are needed for experiments, 10 c.c. of the sodium chloride serum water medium are put into sterile tubes and inoculated with a

few drops of a culture containing *Trichomonas*. This is incubated at 36° C. In twenty-four hours there will be a rich growth of *Trichomonas* and in forty-eight hours the culture will be swarming with them. Over a hundred have been counted in one field (oc. 10, obj. 16 mm.).

In making counts to determine the relative number of individuals present in a culture, it was necessary to determine whether the organisms are evenly distributed throughout the medium. Samples of the culture were taken from the surface by means of a platinum loop and the number of organisms in one field (oc. 10, obj. 16 mm.) were counted. Specimens of the same culture from the bottom of the tube were taken by means of a capillary pipette, and were studied with the same magnification. The count for the organisms on the surface in five fields was: 15, 14, 12, 17, 13; and for those from the bottom of the same tube, 66, 94, 92, 85, 104. Many such counts were made, but the organisms in the bottom of the tube always greatly outnumbered those on the surface. This would seem to suggest that they are dependent on the bacteria which are collected in great masses in the coagulated serum. Further evidence for this was seen when the surface of the tubes occasionally became covered with a growth of bacteria. The *Trichomonas* would then often be found swarming on the surface. As many as 280 and 300 individuals have been counted in fields taken from such a surface.

Sodium chloride serum water was made up in flasks, and, with phenol red as indicator, its reaction was adjusted by the colorimetric method to pH 8.4, 8.2, 8, 7.8, 7.6, 7.4, 7.2, 7, 6.8. The scale for this indicator does not run below 6.8, but two other flasks were made up which had a pH of about 6.6 and 6. This was only approximately estimated by the color, but the fluids were known to be decidedly acid. The adjustment was made by adding hydrochloric acid and sodium hydroxide.

From these flasks 10 c.c. were put into sterile tubes by means of sterile pipettes and each tube inoculated with 5 c.c. of a culture of a pure line of *Trichomonas hominis* which had been grown in a flask to insure a sufficient quantity for inoculating all the tubes from the same culture. Examinations were made after forty-eight and ninety-six hours. The tubes were first shaken to insure an even distribution of the organisms. Then two loops full of the culture were transferred to a slide and the organisms in three fields counted (oc. 10, obj. 16 mm.).

pH.	Number of tubes inoculated	Number of fields examined	Average number organisms in each field of 48-hrs. culture	Average number organisms in each field of 96 hrs. culture
6. (?)	5	15	5.8	0
6.6 (?)	5	15	9.1	0
6.8	4	12	20.4	4.5
7.	4	12	20.	4.3
7.2	2	6	46.	8.2
7.4	2	6	54.5	10.
7.6	2	6	38.8	5.7
7.8	2	6	51.3	0
8.	2	6	87.	1.2
8.2	2	6	20.1	4.7
8.4	2	6	44.1	.2

From the accompanying table it will be seen that *Trichomonas* grows in an acid as well as in an alkaline medium. The most favorable range of reaction for rapid multiplication is pH 7.2 to pH 8.4, with pH 8 as an optimal point. If it is desired to keep the cultures for a longer time, pH 7.2 and pH 7.4 are better. Here the rate of division is slower and consequently the waste products, which are very poisonous to the organisms, do not accumulate rapidly so that the organisms can live longer.

Animal Experimentation. Several authors have reported inoculating animals with *Trichomonas*. Escomel [2] said he infected the rabbit, guinea-pig, cat and dog with *Trichomonas*. Lynch [3] claimed to have infected a white rabbit with *Trichomonas* from a culture tube by injecting them three inches up into the rectum. Three days later he said the rabbit was passing soft brown stools containing many *Trichomonas*, which had not been previously present. Boyd [4] said he infected a rat by feeding it milk which contained a portion of a culture of *Trichomonas* and what he called *Trichomonas* cysts. The *Trichomonas* was not plentiful and he said: "It remains to be ascertained whether infection by feeding results from the flagellates or from the cysts. By analogy the latter would seem probable and my single feeding experiment, taking into consideration the paucity of flagellates present, would tend to support this view." At the present time it is generally recognized that cysts of *Trichomonas* have not been seen and it is very doubtful whether they are ever formed. With this in mind the results of Boyd seem questionable. Recently Pringault [5] fed *Trichomonas* to a cat, guinea-pig, rabbit, and white rat and injected them into the rectum of a kitten. All his attempts at inoculation gave negative results.

With these discordant results in mind it was decided to try infecting cats and rabbits, paying especial attention to preliminary fecal examinations, as it has been shown that many intestinal protozoa appear in the stools at intervals several days apart, and, unless the infected animals are watched for a period of at least four or five days, the parasites which they carry cannot be accurately determined.

Accordingly three young cats were isolated and daily fecal examinations made for seven days. None of them was infected with flagellates. They were then fed, by means of a pipette, two-day-old cultures full of *Trichomonas hominis*. Each cat received from 8 to 9 c.c. of the culture. For six succeeding days the stools were examined microscopically, but *Trichomonas* did not appear and the stools were normal.

Four kittens, which were three weeks old, were next used. The stools were examined for seven days and found not to contain *Trichomonas*, though all were infected with *Giardia*, spirochætes and *Bclascaris*. On the seventh day they were fed 10 c.c. of a culture of *Trichomonas hominis* containing 20-24 organisms in each field (oc. 10, obj. 16 mm.). Daily fecal examinations were made for five days but *Trichomonas* did not appear. Then 10 c.c. of a three-day-old culture were injected into the rectum of each kitten. The rectal tube was passed two and a half inches up into the large intestine. After this, fecal examinations were made for six days with negative results. The kittens were again inoculated *per rectum* with 10 c.c. of a culture containing 25 to 35 *Trichomonas* per field. The succeeding eleven fecal examinations were negative. A third attempt was made to infect them *per rectum*, but fecal examinations covering five weeks did not reveal any *Trichomonas*.

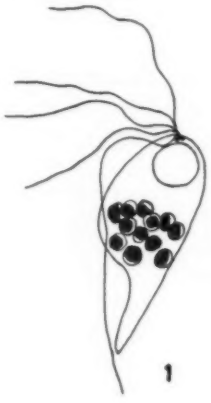
Since both Escomel [2] and Lynch [3] have reported successful inoculation of rabbits with *Trichomonas*, these animals were next tried. Two rabbits were examined daily for nine days and found not to be infected with *Trichomonas*, though one of them was infected with a flagellate closely resembling *Trichomonas* which appeared at irregular intervals, often three days apart. The flagellate did not have an axostyle nor an undulating membrane. It resembled somewhat a *Prowazekia*. It was very plastic. Both rabbits were given *per rectum* 13 c.c. of a rich culture of *Trichomonas*. On eight succeeding days fecal examinations did not show *Trichomonas*, though the other flagellate still appeared at irregular intervals. A second inoculation of 17 c.c. of the culture was given *per rectum*, but the eight succeeding examinations were negative.

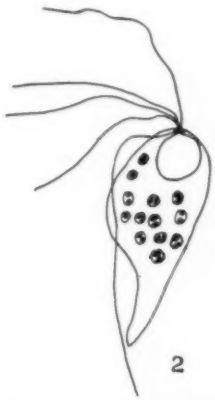
An attempt was made to inoculate two white rabbits, since Lynch [3] especially designated the use of this kind of rabbit. On these animals preliminary stool examinations were made for four days, which were negative. They were then inoculated with 25 and 30 c.c., respectively, of a rich culture of *Trichomonas*. The eight succeeding fecal examinations were all negative.

From the above experiments the *conclusion* has been drawn that cats, kittens, and rabbits cannot become infected with *Trichomonas hominis*. Organisms closely resembling *Trichomonas* were found in the stools of rabbits before and after inoculation. The question is opened whether they have not possibly misled some of the former investigators who claim to have inoculated these laboratory animals with *Trichomonas*.

Vital Stains. These rich cultures of *Trichomonas hominis* offered a good opportunity for studying the effect of vital stains on an intestinal protozoan parasite. Neutral red, methylene blue, brilliant cresyl blue and pyrrol blue were all used in varying dilutions in hanging drops of the culture. The coverslips were sealed with vaseline. Neutral red stained granules in vacuoles which were usually situated in the posterior two thirds of the body (Fig. 1). A few neutral red vacuoles were occasionally seen around the nucleus in the anterior part of the body (Fig. 2). The granules in the vacuoles varied in size and in number, as has been shown to be the case with *Amœba*.[6] Brilliant cresyl blue stained the granules a purplish blue and the vacuole pink. Methylene blue stained the granules blue. Pyrrol blue did not stain any part of the *Trichomonas*.

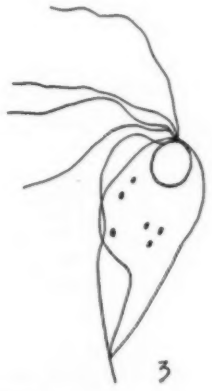
Mitochondria. With janus green the mitochondria stained a blue green. They were short thick rods arranged in groups and singly (Figs. 3, 4). They were not numerous.





The outlines of Figs. 1, 2, 3, and 4 were made with a camera lucida from a prepared slide (×3000 diameters). The neutral red granules in Figs. 1 and 2 and the mitochondria in Figs. 3 and 4 were put in by free hand, as the animals were moving too rapidly to draw with the camera lucida.

Fig. 1 shows large neutral red vacuoles containing single neutral red granules.



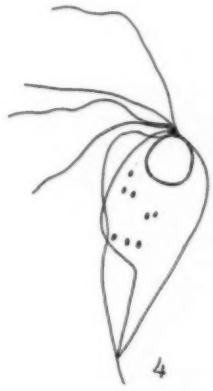


Fig. 2 shows the neutral red vacuoles containing neutral red granules of different sizes. The vacuoles are distributed throughout the body.

Figs. 3 and 4 show the short rod-shaped mitochondria which are scattered about in the body of the *Trichomonas*.

SUMMARY

1. Sodium chloride serum water has proved an excellent medium for the cultivation of *Trichomonas hominis*. When the tubes are covered with paraffin oil the organisms live from 35 to 66 days without being transferred.

2. *Trichomonas* multiplies most rapidly on sodium chloride serum water medium with a pH 8. It lives longest on the same medium with a pH 7.2-7.4.

3. Attempts were made to infect cats, kittens and rabbits with *Trichomonas hominis* but all attempts failed.

4. *Trichomonas hominis* contains granules which take the vital stains, neutral red, methylene blue and brilliant cresyl blue.

5. Mitochondria in the shape of short blunt rods are present and stain with janus green.

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ENTERO-URETHRAL FUSION IN A FETUS, SIMULATING FETAL ASCITES

By JOHN C. BALDWIN

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About 80 cases of congenital fusion of the intestinal and urinary tracts have been described in the literature. These can be divided into a variety of types. In some, the intestine communicated with the bladder, in others, with the urethra. In most instances the junction was in the midline; in many, however, the colon emptied into one side of an asymmetrical bladder. The bladder was dilated in relatively few of the cases. With rare exceptions, the abnormality has occurred in males, but a closely related malformation in the female, in which the rectum opens into the vagina, is quite common. A survey of the literature dealing with the subject revealed no case as young as the one here described and none in which the prostatic urethra participated in the formation of the bladder cavity. Most of the reported cases were those of infants born alive, on whom the diagnosis was made because of the passage of meconium through the urethra in the absence of an anal orifice. Surgeons have made use of the nature of the mixture of urine and meconium to locate the site of the fusion. If the fluid passed at the beginning of micturition was meconium and later clear urine was passed, it was assumed that the colon emptied into the urethra; whereas, if the fluid was a mixture of urine and meconium, then the point of union was assumed to be in the bladder. In either case, the communication was usually so small as to preclude the possibility of satisfactory defecation and to make early operation imperative.

The fetus described in this paper (male, 85 mm. crown-rump length, Carnegie Collection No. 2547) was presented to the laboratory by Dr. N. W. Ingalls, of Cleveland, as a case of fetal ascites. Examination showed a bizarre creature with an enormously distended abdomen which made the extremities appear small by contrast (Fig. 1). The external genitalia were so under-developed as to leave the question of sex in some doubt until after-examination of the gonads. The anal orifice was missing and there was no clear indication of the normal site of the anus. As fetal ascites is characterized by a disproportionately large abdomen, in the absence of peripheral edema this diagnosis was accepted as correct. The existence of an imperforate anus excited no suspicion of the true condition, since one abnormality is frequently associated with others. Furthermore, a dead-born infant with an imperforate anus alone does not, as a rule, show abdominal distention. At autopsy, however, it was found that the abdominal enlargement was due to an abnormally dilated bladder (Fig. 2).

The thoracic viscera showed no gross anomaly. The liver and small intestine were removed and found to be normal. The kidneys were normal in size and position and sections of them showed no dilatation or cyst formation. The sex glands, which lay just below the pelvic brim, proved to be testicles, thus establishing the sex of the fetus. The ureters were not dilated as far as they could be followed behind the large bladder. A section of the bladder wall showed the musculature to be made up of non-striated fibers of the usual bladder type.

Transverse sections 100 μ in thickness were made from a level just below the lower poles of the kidneys downward through the genitalia. These sections were stained with hematoxylin and eosin. The outstanding malformation shown by a study of these sections is an abnormal relation between the descending colon and the urinary tract. The colon opens into the prostatic urethra, which in turn is dilated to such an extent that it has become anatomically a part of the bladder. The bladder, as can be seen from figure 2, is enormously dilated. The wall of the fundus shows little if any hypertrophy, while in the region of the trigone the mucous layers appear to be somewhat thickened and infiltrated by cells with deep-staining nuclei. The posterior wall of the bladder is

FIG. I

FIG. II

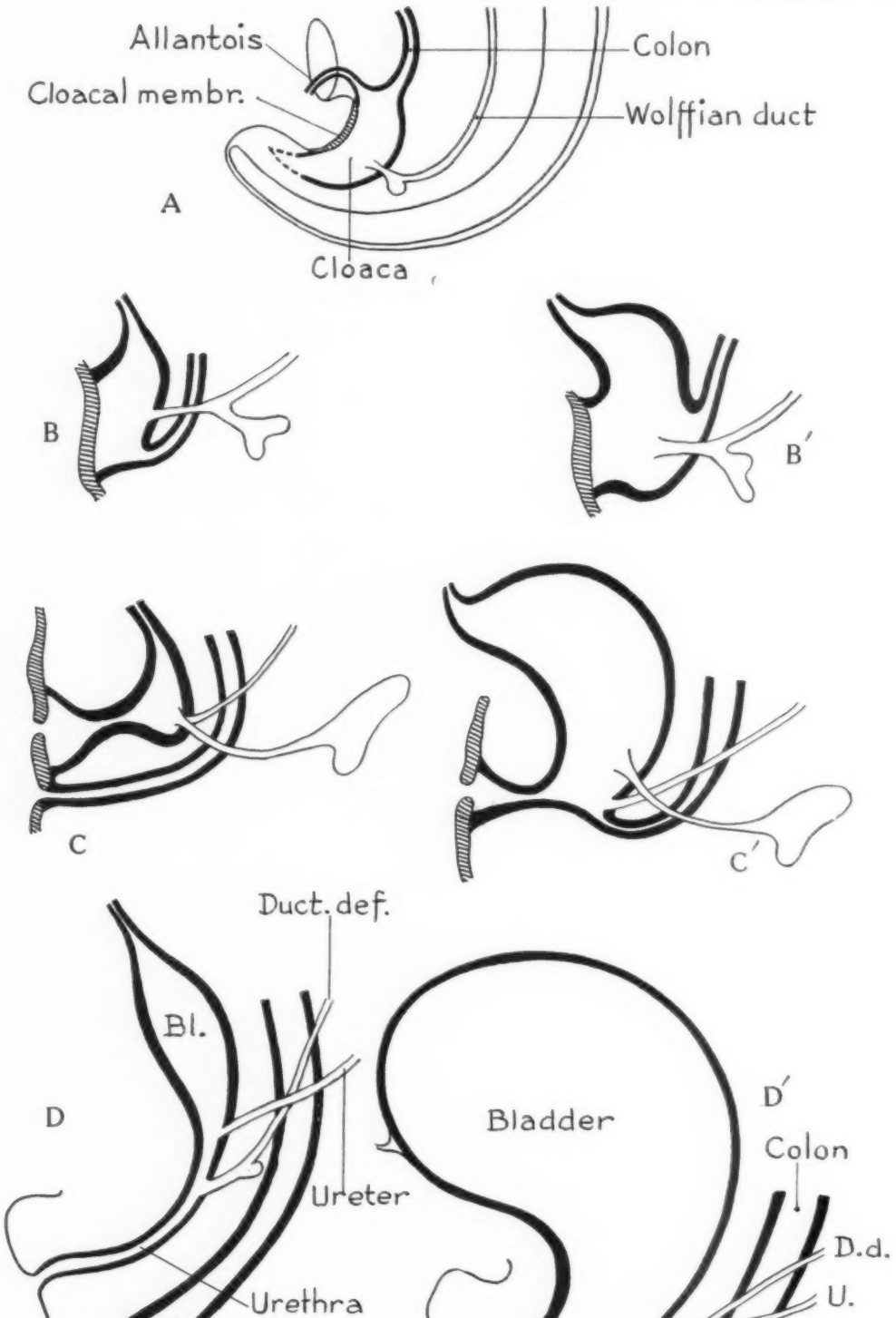
Allantois
Cloacal membr.
Colon
Wolffian duct
A
Cloaca
B
B'
C
C'
Duct. def.
Bl.
D
Ureter
Urethra
Colon
Bladder
D'
Colon
D.d.
U.
Urethra
NORMAL
REPORTED CASE

FIG. 3

folded sharply backward upon itself so as to form a deep longitudinal sulcus, at the base of which the urethra leaves the bladder. Running upward along the posterior wall of the sulcus, from the opening of the urethra, is a band of mucosa strikingly different from that covering the rest of the bladder and resembling closely that of the urethra, with which it is continuous. From this point the urethra can be followed to the external meatus on the rudimentary penis. Its lumen, although filled with cell debris in places, is clearly patent. From the point where it leaves the bladder to the meatus it is not dilated. Normally, the prostatic urethra at this age is relatively wide, with its lumen continuous with the bladder cavity. In this specimen it has widened still further until it has become anatomically a part of the bladder. This appears to account for the band of urethral mucosa found on the bladder wall and is confirmed by the relation which the deferential ducts and seminal vesicles bear to this band of mucosa. The ducts at this age normally join the prostatic urethra a little below the bladder, as has been shown by Johnson.[1] In this specimen they are lost in the bladder wall just lateral to the band of urethral mucosa mentioned above. In other words, that part of the bladder cavity from which the mouth of the urethra opens is not, from a developmental standpoint, bladder at all, but is in reality a dilated prostatic urethra. The membranous portion of the urethra, instead of its prostatic portion, constitutes the exit from the bladder cavity.

The ureters follow a normal course and join the bladder in the usual locations. The right ureter is slightly dilated as it passes through the bladder wall and at its point of entry the bladder is folded upon itself so as to form a pocket.

The descending colon in its upper part appears normal in all respects. As it approaches the posterior wall of the bladder, however, it narrows rapidly until the lumen is barely distinguishable. The muscle layers retain their normal thickness throughout, but the mucosa becomes thin and atrophic as the lumen narrows. This abnormal colon penetrates the posterior wall of the prostatic urethra just above the exit of the membranous urethra from the bladder. No further traces of the intestinal canal are found at lower levels. These relations are shown schematically in figure 3, D′.

DISCUSSION

An interpretation of this condition requires answers to several questions, on some of which our knowledge is inadequate.

What happened to interfere with the usual separation of the urinary and alimentary tracts, and when? The normal transition from the early cloacal phase to complete separation of these tracts is shown in figure 3-A.B. C.D. It will be seen that a wedge of tissue pushes downward, forcing rectum and urethra apart. In our specimen something happened, in the interval between the stages represented by diagrams A and C, to check the descent of this wedge, leaving the colon to empty into the prostatic urethra. In figure 3, D′ represents the condition found in this embryo, while B′ and C′ represent an attempt to bridge the gap between D′ and normal A. As B shows the relations in an embryo of about five weeks, normal development must have been interfered with at about this time, probably in the first half of the second month.

Why did the bladder dilate? The simple answer that it was distended by increased internal pressure is not a satisfactory one. In the first place, there is no evidence that at this age there is any secretion from kidneys or intestine that might dilate the bladder. In the second place, the ureters, colon, and urethra are all patent and open freely into the vesico-urethral cavity. Any increase in the hydrostatic pressure in the bladder, therefore, would have caused fluids to be forced either into the colon and ureters, thus dilating them, or though the urethra, thus relieving the pressure within the bladder. The ureters, however, are not dilated and the colon is actually narrowed. Hence it is clear that the bladder was not distended from increased internal pressure. The only other explanation that presents itself is that the bladder dilated intrinsically and was filled with fluid only in order to avoid a vacuum.

Anders[2] explains dilatation and asymmetry of the bladder in these cases as being due to an overgrowth of that organ, similar in nature to neoplastic growth. There is, moreover, a certain amount of experimental support for the belief that trophic changes in the embryo may cause dilatation in a hollow viscus. Streeter[3] found in the tadpole that marked dilatation had developed in ear vesicles that had been transplanted to abnormal sites.

Finally, what caused the death of the fetus? That the abnormality itself was the cause is highly improbable, as there is nothing about it incompatible with fetal or even postnatal life. Two other possibilities present themselves. First, that the ovum itself was originally defective and that this defect caused both the abnormal development and the death of the fetus. Second, that the original germplasm was healthy, but that some process, perhaps a toxemia on the part of the mother, so profoundly influenced the embryo as to cause the abnormalities and later its death. Between these possibilities there is no ground on which to base a decision.

I wish to express my sincere thanks to Dr. G. L. Streeter for his many helpful suggestions in the pursuance of this study and for his kindness in extending to me the facilities of the Carnegie Laboratory of Embryology. I also wish to thank Mr. Charles Miller for the preparation of the serial sections.

SUMMARY

A fetus is described, the external appearance of which provoked a diagnosis of fetal ascites.

The abdominal enlargement is due to an enormously dilated bladder. The prostatic urethra is so dilated as to form part of the bladder cavity.

The descending colon empties into the prostatic urethra.

The dilatation of the bladder is thought to be due to overgrowth of bladder tissue rather than to distention.

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DESCRIPTION OF FIGURES

Fig. 1.—Embryo erroneously diagnosed fœtal ascites, showing large abdomen. Carnegie Collection No. 2547.

Fig. 2.—Same embryo with abdominal wall folded back, and liver and intestines removed to show large bladder.

Fig. 3.—*A*. Early relations of colon and urinary tract drawn from model of normal fœtus of 5.5 mm. greatest length. Carnegie Collection No. 1380. *B*. Relations in normal fœtus of 7 mm. From Felix,[4] Fig. 577A. *C*. Relations in normal fœtus of 11 mm. *Ibid*. Fig. 604A. *D*. Relations in normal fœtus of 85 mm. From dissection. *B'* and *C'* represent stages in the reported case corresponding to *B* and *C*, inferred from Normal *A*, and relations as found in *D'*. *D'*. Relations in reported case as established by serial sections.

REPORT OF A CASE OF TUBAL PREGNANCY PROBABLY CAUSED BY A PAROVARIAN CYST

By LEO BRADY, M. D.

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Although it has long been known that tubal pregnancies can be caused by any factor that interferes with the course of the fertilized ovum as it travels inward along the Fallopian tube, this case presents several rather unusual features which seem to justify its publication.

The brief summary of the case is as follows:—F. B. Gyn. No. 154077. Age 26—white—female. Admitted: February 24, 1922.

Family and general past history unimportant. Menstrual history normal until the onset of present illness. Marital history: Married one year, one child born September 1921, that is, five months before the present admission to the hospital, when the patient was still nursing it.

Present Illness.—Three months after the birth of her normal child, that is, two months before admission to the hospital the menstrual period returned. Five weeks later a second period of normal duration occurred. Three weeks later, that is, sixteen days before admission to the hospital, she began to bleed again, although the menstrual period was not due until a week later. The bleeding was not of greater quantity than usually occurred with the periods, but instead of stopping at the end of four or five days continued for sixteen days until the patient was admitted to the hospital. Two days before admission to the hospital the patient felt "generally run down" and went to her doctor who prescribed calomel and salts. The next day she was taken suddenly with a drawing pain over the whole lower abdomen which was so severe that it compelled her to go to bed. The pain was especially bad at stool. She says she felt as if she were going to die. The pains became more severe during that day and the following night. The next day the attending physician noticed the facial pallor, localized the abdominal tenderness in the lower abdomen and sent the patient into the hospital with a diagnosis of ruptured tubal pregnancy.

Admission Note.—The patient is a well developed white woman in extreme prostration, but mentally oriented, complaining of intense dryness of the mouth, weakness and abdominal tenderness. Mucous membranes quite pale. Temperature 98.4°; pulse 140, weak and irregular. Respirations 28, white blood count 15,000. Red blood count 2,160,000. Hemoglobin 33%. Blood pressure, 96/74. General physical examination essentially negative. The breasts are swollen and tender and contain milk. General abdominal tenderness is present. Shifting dullness is demonstrable in the flanks. Pelvic examination: Outlet marital, mucous membranes very pale. A slight amount of bloody vaginal discharge. Cervix soft. Filling the whole cul-de-sac there is a soft mass which to the examiner has the characteristic consistency of blood-clots.

Pre-Operative Diagnosis.—Ruptured extrauterine pregnancy.

When the patient was first seen, it was at once evident that she could not in her condition stand an operation and, unless something were done very soon, she would die. The husband's blood fortunately matching with hers, a transfusion of 600 c.c. of citrated blood was given in the operating room. Although the patient had a slight chill and a rise of temperature after it, her general con-

Left parovarian cyst
Tube
Ovary
Fimbr. extr.
Parovarian cyst
Max Brödel
1922

dition improved at once and the blood pressure rose from 96 to 120. One hour after completion of the transfusion the operation was begun. After division of the fascia and separation of the muscles, the blood-clots filling the abdominal cavity could be seen shining through the peritoneum, and when the peritoneum was opened, there was an outpouring of a large quantity of blood. The entire pelvis was filled with blood, and blood was seen pouring from a rupture which had occurred in the right Fallopian tube at a distance of about 2 cm. from its fimbriated end. There was a small parovarian cyst present which had kinked the tube just proximal to the point where the tubal pregnancy had occurred and which was in all probability the causal factor of its development. On the left side there was also a parovarian cyst, but this was much larger, measuring 12 cm. in diameter. The tube on the left side was drawn over the cyst. The ovaries on both sides were perfectly normal. I removed the Fallopian tube on the right with the extrauterine pregnancy, both parovarian cysts and as much of the blood-clots from the adbominal cavity as I could without prolonging the operation. At the termination of the operation the pulse was 160 but of good quality.

Convalescence was quite satisfactory and the patient was discharged in good condition twenty-two days after her operation.

Pathological Report (Dr. Warrinner).—Gyn. Path. No. 27577.

The right tube measures 8 by 2.5 cm. in its distal third. The tube is ruptured 1.5 cm. from its fimbriated end. Just under the point of rupture is a parovarian cyst. This cyst is thin-walled and filled with clear fluid. In the center of one of the large blood-clots removed at operation the fetus and membranes are found. The fetus measures 5 cm. in length.

In addition to being one of the many cases we now see of tubal rupture with complete collapse of the patient, in which a transfusion improves the condition sufficiently to allow the performance of a successful operation, this case presents the two following interesting factors:

(1) This patient developed a tubal pregnancy only five months after the termination of a normal pregnancy and while she was still nursing her baby. (2) It is seldom that one can demonstrate so clearly a mechanical factor causing the arrest of the fertilized ovum in the tube as is shown in this instance.

For the excellent drawing of the conditions found at operation I am greatly indebted to Mr. Max Broedel.

LEGEND

Posterior view of pelvic conditions as found at operation. On the right is shown the parovarian cyst which had kinked the tube at a point immediately proximal to the site of the rupture. On the left the larger parovarian cyst is shown with the elongated and thinned out Fallopian tube extending over its top. The small drawing in the upper right hand quadrant indicates the mechanism by which the right tube has been compressed by the parovarian cyst.

THE ISOLATION OF NUCLEIC ACID FROM TISSUES

By WALTER JONES AND CASPAR FOLKOFF

(From the Laboratory of Physiological Chemistry, Johns Hopkins Medical School)

The well known chemical distinction between animal and plant nucleic acid suggests an inquiry into the chemical nature of bacterial nucleic acid to learn whether in this respect bacteria are to be regarded as animals or plants. Attempts to decide this question failed for want of a method of isolating nucleic acid that is applicable to the material which constitutes the bodies of bacteria, until recently, when one of us (Folkoff), in collaboration with Schaffer and Bayne-Jones,* prepared a considerable amount of bacterial nucleic acid and found the substance somewhat unique. The method of isolation employed by these experimenters had been previously elaborated by us in connection with yeast and is here described for purposes of reference.

Five liters of cold fresh brewer's yeast diluted with eight liters of cold tap-water are treated with 2½ liters of 20% sodium hydroxide in small successive portions, and the mixture is stirred continually for ten minutes, care being taken that the temperature of the material does not rise. At the end of this time most of the alkali has been neutralized with hydrochloric acid. The solution is finally made faintly but distinctly acid to litmus with acetic acid and after standing over night in a cold place the muddy opalescent dark brown fluid is decanted from the sharply settled yeast detritus and treated with hydrochloric acid and alcohol in the usual way for the precipitation of the nucleic acid, which is washed by decantation with alcohol of increasing strength and finally filtered tightly on a Buchner funnel.

The crude nucleic acid thus obtained is ground in a mortar with five or six parts of water containing an excess of ammonia, and after the solution of the nucleic acid is complete, an equal volume of alcohol is added. The excess of ammonia is then carefully neutralized with acetic acid. This is the crux of the method. *The excess of ammonia must not be neutralized until after the alcohol has been added,* and the end-point of the neutralization is determined not with an indicator but by the

* Schaffer, Folkoff and Bayne-Jones: Johns Hopkins Hospital Bull., 1922, XXXIII, 151.

appearance of a bulky dark brown precipitate leaving an easily filterable interstitial liquid that is as transparent and nearly as colorless as distilled water.

The clear fluid is filtered off, treated with an equal volume of alcohol and the precipitated snow-white flakes are washed and dried with alcohol. As this process of purification does not involve an appreciable loss of material, it may be repeated as often as is suggested by the appearance of the final material, and can be applied to the purification of any of the commercial preparations of yeast nucleic acid.

The final product (30 grams of which are obtained from five liters of yeast) consists of a colorless biuret-free amorphous powder which dissolves easily and completely in water to form a colorless solution. It yields decomposition products which bear the quantitative relation to one another that is required for the ammonium acetate addition product of yeast nucleic acid and contains exactly half of its phosphorus in easily split form, showing that its purine and pyrimidine groups are present in equivalent amounts.

As the method described cannot be applied to the isolation of nucleic acid from animal glands, its successful execution in a particular case would suggest that the questionable material under examination is of plant origin.

A CASE OF CONGENITAL OSTEOSCLEROSIS

By Ralph K. Ghormley, M.D.
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(*From the Orthopedic Service of the Surgical Department of the Johns Hopkins Hospital*)

The following case is presented because of its unique characteristics and in the hope that, if others are recognized, they will be brought to the knowledge of the profession.

Case History

J. C. (Surg. No. 55907), male, while, *æt.* 8, was first seen in the Orthopedic Dispensary of the Johns Hopkins Hospital on February 1, 1922.

Complaint: "Pain in left hip and limp."

F. H. The father and mother are living and well. There is one half brother and one half sister by a former husband of the mother. Since her marriage to the father of the patient there has been one child born prematurely, who lived two months; then there were five miscarriages all before the end of the fourth month. The patient is the last child born. There is no history of either syphilis or gonorrhœa in the father or mother.

P. H. Premature birth at eight months. Weighed four pounds. Breast fed. The child developed quite normally, however, and aside from varicella, at the age of three years, there have been no acute illnesses. Tonsillectomy and adenoidectomy were performed one year ago. The mother states that two years previous to the present illness the child had some "growing pains" in the back for a short time.

P. I. During the first week in December, 1921, the boy complained of pain in the left hip which was more marked at night and toward the close of the day. This pain would also be noticed after resting from his play. The parents noticed a slight limp. There was no history of any injury. There was never any swelling and no night cries. The condition has not progressed to any appreciable extent.

Physical examination. Blood pressure, 100/65; pulse, 80; respiration, 18; temperature, 99.2; Height, 4 ft., 3 in.; weight, 58 lbs.

The patient is a fairly well developed and nourished boy; he is bright and active. There is no suggestion of any mental retardation (Fig. 1).

Head: Negative.

Neck: Negative, thyroid not palpable.

Chest: Heart and lungs clear, no retro-manubrial dullness, no rachitic rosary.

Abdomen: The abdomen is rather prominent. The walls are everywhere soft. There is no tenderness anywhere and no masses are felt. None of the organs are palpable.

Glands: There is some enlargement of the posterior cervical lymph glands which are discrete and not tender. The axillaries and epitrochlears are not felt. The inguinals are moderately enlarged, discrete and not tender.

Skeletal System. Spine: The spine is quite straight, although there is slight deviation toward the right to compensate for the slight tilting of the pelvis toward the left. The movements of the spine are free in every direction and there is no tenderness anywhere. *Upper extremities:* The muscular development is good. There is no bony tenderness or thickening. The joints are all normal. *Lower extremities:* The child walks with a moderate limp toward the left. The left foot is held rotated outward when standing. There is slight atrophy of the thigh and calf on the left. When he lies down, there is about half a centimeter of apparent shortening of the left leg. Voluntary as well as passive movements of the left hip reveal slightly less abduction than on the right. There is a slight decrease in the hyperextension of the hip. There is no muscle spasm and no tenderness. The measurements are:

	Right	*Left*
Apparent shortening		0.5 cm.
Ant. sup. spine—Int. malleolus	64 cm.	63.5
Great trochanter—Ext. malleolus	61.5	61.5
Base of Bryant's triangle	4.5	3.75
Circumference of thigh	33.5	32.5
Circumference of calf	24.5	24.

Blood. Hemoglobin 70% (Sahli)
R. B. C. 4,600,000
W. B. C. 7,050

Differential count:

P. M. N.	56.3%
P. M. E.	.33
P. M. B.	.33
L. Lymph.	2.33
S. Lymph.	36.3
L. Mono.	.66
Trans.	3.66

There is slight anisocytosis and poikilocytosis. Hemolysis resistance tests are normal.

Platelet count, 128,000.

Reticulated R. B. C., 0.9%.

Blood Wassermann, negative; ice-box preparation, negative.

Blood Chemistry:

N. P. N., 30 mgm. per 100 c.c.
CO_2, 47.1 vols %
Sugar, .119
Phosphorus, 4.8 mgm. per 100 c.c.
Calcium, 10.2 mgm. per 100 c.c.

The blood culture showed no growth.

Urine: Sp. Gr. 1018; acid; albumin and sugar tests negative; microscopic examination negative.

Basal Metabolism, 11.1% above the average for the age and sex.

During the course in the hospital, while under observation, there was an almost daily afternoon rise in temperature to about 100° F. Nothing was found to account for this. Operation was refused by the parents, so no exploration could be carried out. A plaster of Paris spica was applied with the leg as fully abducted as possible without anesthesia. This was worn for about three weeks after which time a radiograph showed little change in the hip. The boy, however, had no further pain and the limp seems to be less.

DISCUSSION

On seeing the patient in the dispensary our impression was that we were dealing with a beginning epiphyseal separation of the head of the femur on the left. Radiographs of the pelvis taken at that time showed a curious dense appearance of the bones. This was thought at first to be an artefact, but carefully repeated pictures showed the same condition (Fig. 2). Radiographs of all of the bones were then made and showed much the same condition throughout the skeleton. Beside the curious density of the flat bones and the rather hazy outline along all of the bones, the vertebral bodies showed a marked density at either pole (Fig. 3). There is thickening of the cortex of the ribs (Fig. 4), the marrow cavity in places being apparently obliterated. There is definite thickening of the cortex of all the long bones, more marked in the femora and humeri (Figs. 5 and 6), and the cortical thickening seems to be greater in the proximal portion of these bones than in the distal portion. The skull (Fig. 7) is thicker than normal. In the femoral necks, and especially in the left, there seems to be a breaking down of the bone just below the epiphysis so that the epiphysis is slipped inward and downward (Fig. 2).

Radiographs of the mother and father were then made, which showed the mother's bones to be normal but that practically the same condition existed in the father as in the child (Figs. 8 and 9). The father's history was then reviewed and it was found that both of his parents had lived to be eighty and that he has seven brothers, all of whom are living and in good health. It is hoped that we may be able later to get radiographs of some of these brothers. The father states that when he was a boy he had "growing pains" in his legs, but had no treatment and entirely overcame them after adolescence. The blood Wassermann of the father, as well as of the mother, was negative. Smears of the father's blood were normal. No dietary discrepancy in either father or son could be discovered.

A search through the literature has failed to locate any cases similar to this in the living person. There are, however, several cases described at autopsy as osteosclerosis, some as congenital and others as associated with disturbance of the blood-forming organs.

Frangenheim [1] has collected three cases from other writers of autopsy reports of congenital osteosclerosis. Two of these were in infants with a normal blood picture but a third, a case reported by Goodall [2] was in a child who died at the age of ten weeks of what is described as acute myelocythæmia, of three weeks' duration, with bleeding from the mucous membranes, enlargement of the liver and spleen and a blood picture of myeloid leukæmia. Beside the changes in the organs the bones showed osteosclerosis of the ribs, sternum, femora, humeri and tibiæ. Another of these cases was originally reported by Schmidt of a child who died after thirty hours with enlarged thymus, normal blood smears and an apparently normal bone-marrow. The bones showed marked thickening of the cortex of the long bones, skull and sternum and a marked density in the vertebral bodies. The other case cited by Fragenheim was originally reported by Assmann. A child died, when one day old, showing beside enlargement of the thymus and thyroid a normal blood picture and normal bone-marrow. There was an associated diffuse sclerosis of the long bones as well as of the skull and vertebræ. In all of these cases the cortical thickening was mostly central with partial obliteration of the marrow cavities, there being some cartilaginous rests and the marrow in places being encased in thick-walled bony spaces.

Assmann [3] has collected in addition four cases in adults who died at ages ranging from 17 to 68 years.

Two died of leukæmias and the others, it was thought, of primary anæmias. All of these cases showed similar bone changes at autopsy.

Neuwerck and Moritz [4] report one case with similar bone changes but draw no conclusions except to refute the statement of Schlazenhofer [5] that the osteosclerotic process may be due to arsenic or phosphorus used in the medication. Neither of these drugs had been used in their case.

Schwarz [6] reports a case of leukæmia with osteosclerosis in a woman, 47 years old, who died after a six months' illness, the autopsy showing, beside enlargement of the liver and spleen with findings characteristic of leukæmia, a thickening of the cortex especially of the femora and humeri. The ribs, vertebræ and skull also showed sclerosis. In the long bones of this case it is noted that the proximal portion of the cortex was more markedly thickened than the distal portion. The marrow cavity is described as grayish red to dark brown in color with bony trabeculæ scattered through the marrow.

Heuck [7] in autopsies on two cases of leukæmia in men, 24 and 25 years of age, found marked thickening of the diaphyses of the femora and humeri, as well as of the vertebræ and skull. He holds that the osteosclerosis is of long standing and that the blood changes are due to the changes wrought in the marrow by the osteosclerotic process, or that the occurrence of the two is a coincidence.

The above case reports are all pathological without any radiographs. However, the anatomical descriptions are so nearly what one would expect to find at autopsy in the case here reported that we feel we are dealing with the same condition. The majority of the authors take the view that the sclerosis is secondary to an irritation which goes with the morbid process in the blood. Heuck, however, takes an opposite view, as above stated. In our case we seem to be dealing with a definitely inherited condition not related, so far as we know, to any metabolic disturbance which we can demonstrate. There is possibly some increase in the blood phosphorus, the figures given being above the average normal for the age and sex. There is, however, no evidence of either the father or son having been on a high phosphorus diet. It would seem that the condition itself is not incompatible with life and that the secondary blood diseases may come as a coincidence or as a failure of the decreased blood-forming mechanism to respond to the excessive demands placed upon it.

We are greatly indebted to the various laboratories, for aid in the investigations in this case.

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BOYLSTON MEDICAL PRIZES

These prizes, which are *open to public competition*, are offered for the best dissertation on questions in medical science proposed by the Boylston Medical Committee.

At the annual meeting held in Boston in 1920 a prize of three hundred dollars was awarded to an essay entitled "Acute Inflammation of the Nose, Pharynx and Tonsils" by Mr. Stuart Mudd of St. Louis.

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In awarding these prizes, preference will be given to dissertations which exhibit original work, but if no dissertation is considered worthy of a prize, the award may be withheld.

Each dissertation must bear, in place of the author's name, some sentence or device, and must be accompanied by a sealed packet, bearing the same sentence or device, and containing the author's name and residence within.

Any clew by which the authorship of a dissertation is made known to the Committee will debar such dissertation from competition.

Dissertations must be printed or typewritten, and their pages must be bound in book form.

All unsuccessful dissertations are deposited with the Secretary, from whom they may be obtained, with the sealed packet unopened, *if called for within one year* after they have been received.

By an order adopted in 1826, the Secretary was directed to publish annually the following votes:

1. That the Board does not consider itself as approving the doctrines contained in any of the dissertations to which premiums may be adjudged.

2. That, in case of publication of a successful dissertation, the author be considered as bound to print the above vote in connection therewith.

The address of the Secretary of the Boylston Medical Committee is REID HUNT, M.D., Harvard Medical School, Boston, Mass.

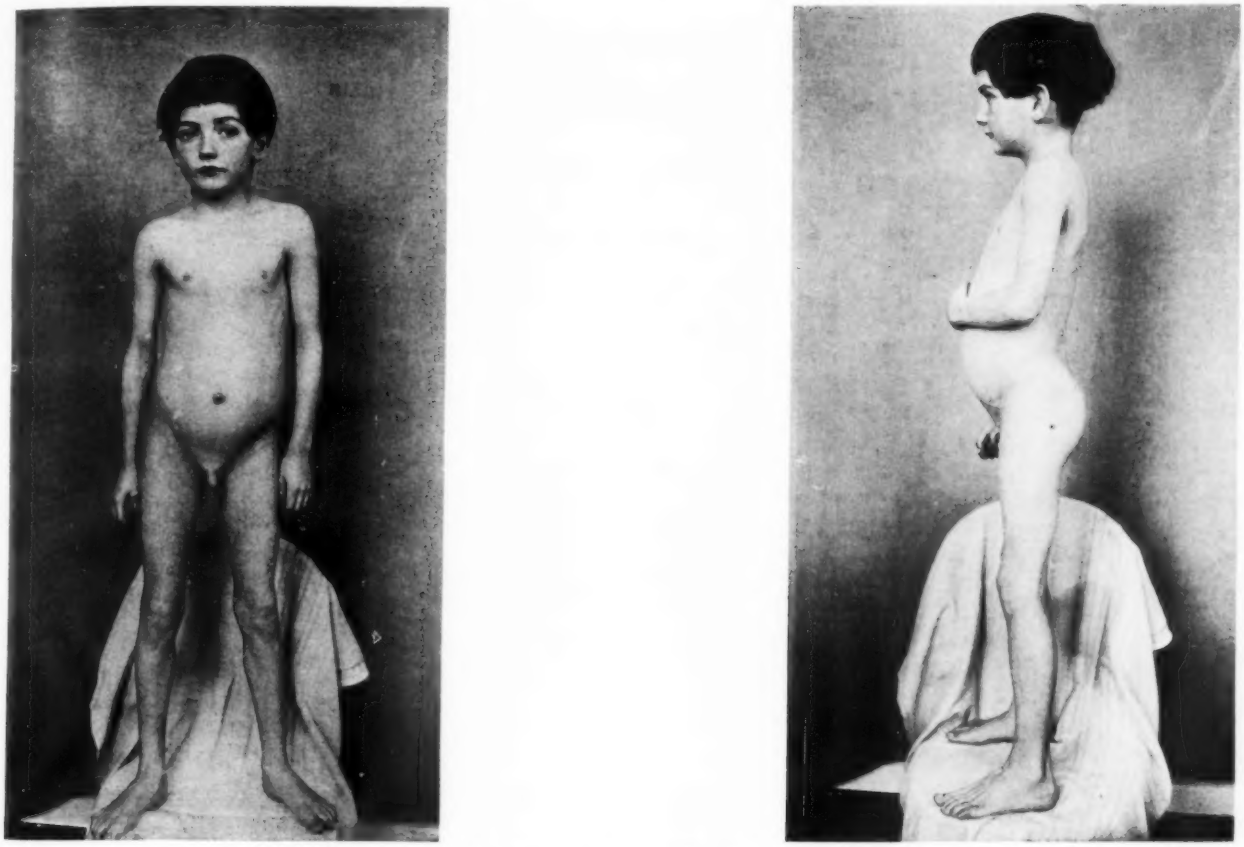

Fig. 1.—Showing distortion and deformity, the result of coxa vara.

Fig. 2.—Osteosclerosis of all the pelvic bones with partial subluxation of the epiphyses of the femora and some new bone formation around the epiphyseal lines.

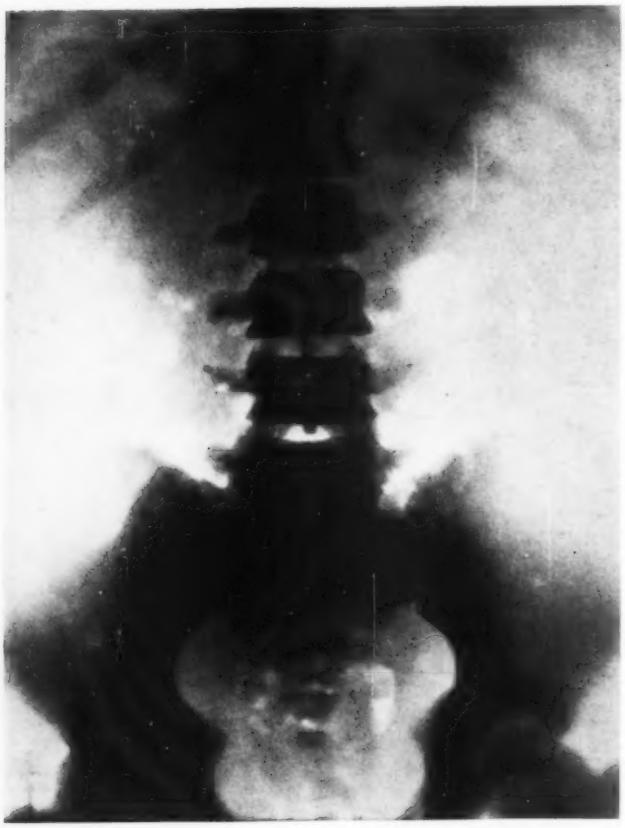

Fig. 3.—Showing marked osteosclerosis of the poles of the vertebræ.

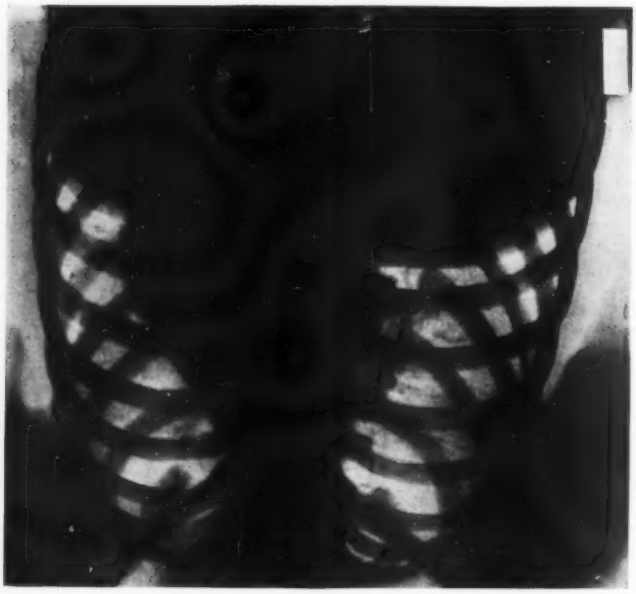

Fig. 4.—Showing thickening of the cortex of the ribs.

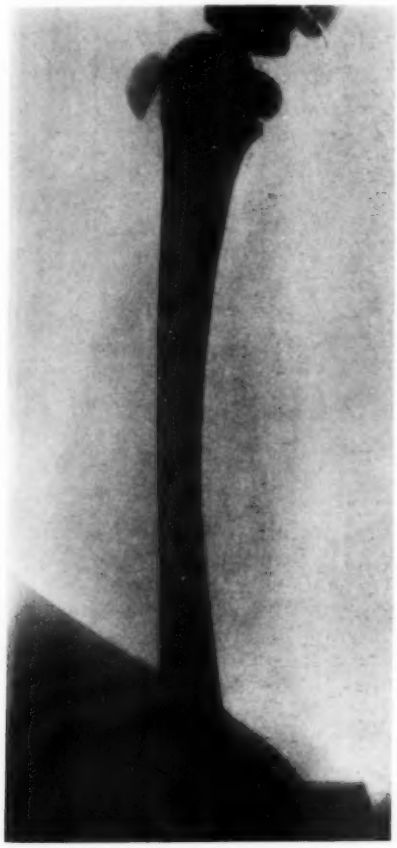

Fig. 5.—Showing a marked thickening of the cortex.

Fig. 5a.—Showing a marked thickening of the cortex.

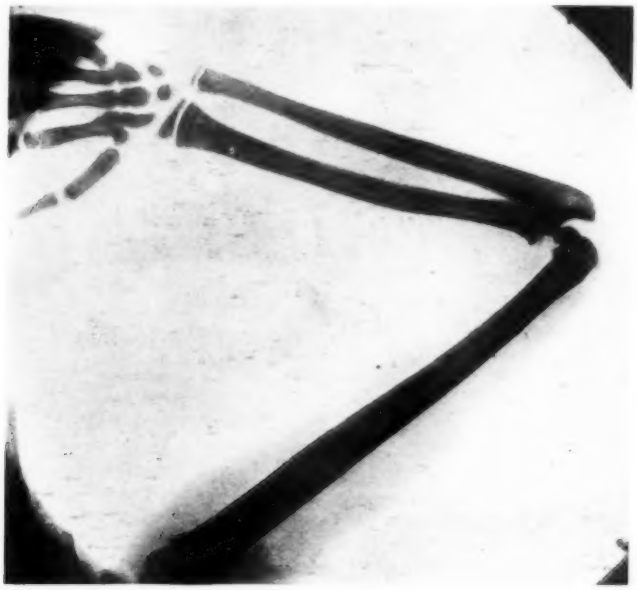

Fig. 6.—Showing a marked thickening of the cortex.

Fib. 7.—Marked osteosclerosis of the bones at the base of the skull.

Fig. 8.—Osteosclerosis of the pelvic bones of patient's father. These show no subluxations of the epiphyses.

Fig. 9.—Spine of patient's father, showing the same osteosclerotic condition of the poles of the vertebræ.

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ERRATA

Attention is called to two mistakes in the article by Dr. J. Whitridge Williams entitled "The Influence of the Treatment of Syphilitic Pregnant Women upon the Incidence of Congenital Syphilis" which appeared in the November Bulletin.

On page 386, second column, the last paragraph should read as follows:

In *conclusion,* I am aware that the present paper is based upon too small a material to possess any statistical value, but at the same time the results which we have obtained are highly suggestive and are interesting from two main points of view. First, because our study is based upon the observations made upon a series of women with whose past history we have long been familiar, and secondly, because the results obtained are extraordinarily stimulating from the standpoint of treatment and of the great hope which it offers for the future.

On page 385, Table II, the total for "Deaths after Treatment" should be 9 instead of 3.